JOURNAL OF AGRICULTURAL RESEARCH

VOL. 50 WASHINGTON, D.C., MAY 1, 1935 No. 9

RATE OF DECOMPOSITION OF ORGANIC MATTER IN NORFOLK SAND AS MEASURED BY THE FORMATION OF CARBON DIOXIDE AND NITRATES[1]

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INTRODUCTION

Norfolk sand is extensively found throughout the South and is the predominant type of soil in Florida (*2*).[3] Because of its physical properties and the climatic conditions of Florida, this type of soil is not adapted for retaining organic matter, the addition of which, together with commerical fertilizers, is necessary for the production of maximum crops.

As this study was made in connection with a soil-building program it was thought advisable to study the comparative rate of decomposition of various organic materials in Norfolk sand, under different environmental conditions, with and without the addition of inorganic fertilizers and in the presence and absence of citrus seedlings. Several types of organic materials common to Florida were used, including both legumes and nonlegumes. Leukel, Barnette, and Hester (*3*) studied the rate of decomposition of various parts of *Crotalaria striata*, one of the plants used in the present work.

EXPERIMENTAL MATERIALS AND METHODS

The materials used in these experiments were stable manure and the plants Crotalaria (*Crotalaria striata*), beggarweed (*Desmodium tortuosum*), cowpea (*Vigna sinensis*), and Natal grass (*Tricholaena rosea*). The plants were at the seedling stage; their composition is given in table 1. The entire plant, including roots, was dried in an oven at 65° C. in order that the material might be finely ground and thus insure a more uniform mixture with the soil. These dried organic materials were added to the soil at the rate of 1 percent by weight.

Norfolk sand (deep phase) was used throughout the investigation, and all soil was air dried before the materials were mixed with it and placed in the pots.

[1] Received for publication Dec. 5, 1934; issued July 1935. Part of a thesis presented to the graduate faculty of the Iowa State College of Agriculture and Mechanic Arts in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] During the course of this investigation the author has discussed his problems with Drs. P. E. Brown and F. B. Smith of the Iowa State College, and Drs. R. W. Ruprecht and R. M. Barnette of the Florida Agricultural Experiment Station. For their helpful suggestions and criticisms he is grateful.

[3] Reference is made by number (italic) to Literature Cited, p. 730.

TABLE 1.—*Loss on ignition and composition (percent) of organic matter added to the soil*

Material	Loss on ignition	SiO_2 and sand	N	CaO	MgO	P_2O_5	Na_2O	K_2O
Cowpeas	93.53	1.93	2.44	0.78	0.44	0.85	0.23	0.91
Crotalaria	95.51	.65	2.37	.50	.33	.86	.20	1.24
Beggarweed	95.33	1.18	1.66	.62	.32	.44	.27	.92
Natal grass	89.29	7.61	.71	.16	.27	.74	.20	.80
Manure	37.06	54.91	1.01	.33	.33	2.05	.32	1.34

The additions of all inorganic salts were calculated on a basis which would supply plant food equivalent to that contained in 100 pounds of a 4–8–4 commercial fertilizer per acre of 67 trees, or an average of 1.5 pounds per tree. The quantity of inorganic salts added to each pot was in the same relative proportions, per unit area, as the quantity (1.5 pounds) spread around a 1-year-old citrus tree under field conditions. By using 1.5 pounds per tree as a basis, the amounts of inorganic salts required per pot were as follows:

Complete fertilizer { 4.79 g. ammonium sulphate; 13.31 g. superphosphate; 2.49 g. potassium sulphate }

All pots to which ammonium sulphate alone was applied received this material in the same quantity that it was used in the complete fertilizer.

The containers used in the experiment were ordinary 4-gallon, glazed, earthenware pots with a ¾-inch hole in the side near the bottom. This hole facilitated drainage and allowed air to be drawn through the soil when the carbon dioxide in the soil air was to be measured.

To 40 pounds of air-dried soil the materials shown in table 2 were added.

TABLE 2.—*Quantities (grams) of material added to 40 pounds of air-dried soil in the various pots*

Pot no.	Material added	Quantity added	Pot no.	Material added	Quantity added
1	Crotalaria	181.44	10	Cowpeas	181.44
2	Beggar-weed	181.44		$(NH_4)_2SO_4$	4.79
3	Natal grass	181.44	11	Manure	181.44
4	Cowpeas	181.44		$(NH_4)_2SO_4$	4.79
5	Manure	181.44	12	$(NH_4)_2SO_4$ (check)	4.79
6	Check, no treatment		13	Crotalaria [1]	181.44
7	Crotalaria	181.44	14	Beggar-weed [1]	181.44
	$(NH_4)_2SO_4$	4.79	15	Natal grass [1]	181.44
8	Beggar-weed	181.44	16	Cowpeas [1]	181.44
	$(NH_4)_2SO_4$	4.79	17	Manure [1]	181.44
9	Natal grass	181.44	18	Complete fertilizer only (check)	
	$(NH_4)_2SO_4$	4.79			

[1] Plus complete fertilizer.

The materials were weighed out, thoroughly incorporated in the soil, and placed in pots. There was a total of 72 pots, each treatment being made in quadruplicate. Two of these series (36 pots) were kept fallow while the other two (36 pots) were set with two seedlings of *Citrus* (*Citrus aurantium* L.). One fallow pot of each treatment (18 pots) was placed in the greenhouse under somewhat

controlled temperature and moisture conditions, while the other fallow pot of each treatment (18 pots) was placed outside the greenhouse under ordinary atmospheric or field conditions. One pot of each treatment set to citrus seedlings was likewise placed in the greenhouse and one outside. By following this method it was possible to have two series of the soil treatments (as outlined above) in the greenhouse and two outside, one each of these series of 18 pots fallow and one series set with citrus seedlings. After all the seedlings were set, water was added to the pots in sufficient quantity to bring the moisture content up to 50 percent of the water-holding capacity of the virgin soil. This percentage of water (added as tap

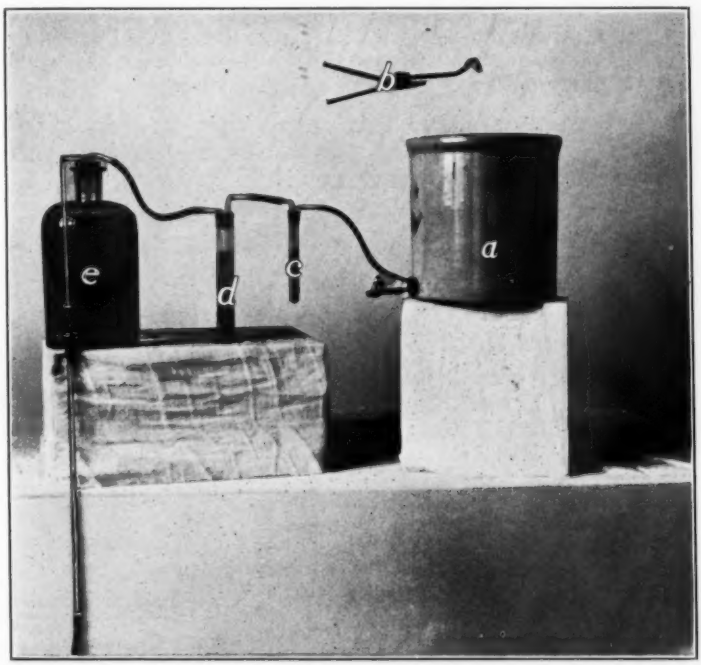

FIGURE 1.—Detail of a single unit of the apparatus used in collecting carbon dioxide: *a*, Soil pot; *b*, tube used for collecting the carbon dioxide and drainage; *c*, test tube containing N/10 solution of hydrochloric acid; *d*, 100-cc absorption cylinder containing 2N sodium hydroxide; *e*, 5-l aspirator bottle filled with water.

water) was maintained throughout the experiment in all pots kept in the greenhouse.

The method used for collecting the carbon dioxide was very similar to that employed by Turpin (*6*). Before the pots were filled with soil a 2-hole rubber stopper was fitted into the hole on the side of the pot near the bottom. Two copper tubes were inserted through the stopper, one to be used for collecting drainage water and the other for drawing air through the soil to displace the soil air. The tube for collecting the carbon dioxide was bent in gooseneck shape with the short bend turned down (fig. 1, *b*) and was long enough to

reach to the center of the pot. Over these tubes fine rocks were placed in order to give good drainage channels and prevent the tubes from becoming clogged with soil. The soil was placed on these rocks. For collecting the carbon dioxide a special apparatus was used which would allow 5 l of the air to be drawn through the soil in each pot. Figure 1 shows a single unit of the set-up used in this work. Two glass receptacles were placed between the bottle and the pot. These receptacles consisted of an 8-inch test tube and a 100-cc cylinder. A 2N sodium hydroxide solution was placed in the cylinder to absorb the carbon dioxide. The test tube containing N/10 solution of hydrochloric acid was placed nearest the pot. To collect the carbon dioxide a 5-l aspirator bottle was filled with water, the hook-up completed, and the overflow regulated so as to allow the aspirator bottle to be emptied in 1 hour; then the flasks were taken to the laboratory for the analyses. A battery of 10 of these units was run simultaneously. The carbon dioxide was collected at

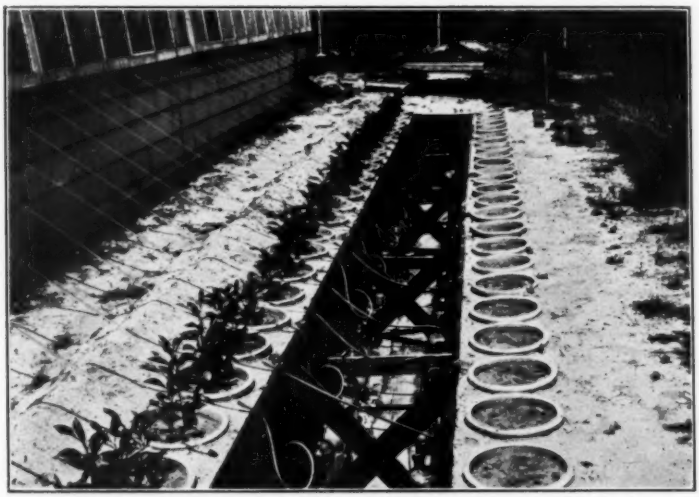

FIGURE 2.—Set-up of experiment outside the greenhouse.

definite intervals and the quantity determined by the use of the double titration method (*1*).

All pots outside the greenhouse were buried in the soil to within 1 inch of the top and so arranged as to allow the copper tubes to project through a wall into a pit. The short tubes were bent down and connnected with bottles for collecting the drainage water. The tubes for collecting carbon dioxide were connected with the collecting units in the greenhouse by the use of long glass tubes. When the soil was being aerated, the drainage tubes were closed, thereby forcing the air through the soil and displacing the soil air. Figures 2 and 3 show the arrangement of these pots outside and inside the greenhouse.

In determining the carbon dioxide content of the soils 22 separate collections were made. The results thus obtained on different dates were averaged by means of a moving average. In the use of the moving average the quantities of carbon dioxide for three consecutive determinations were added and divided by three. The average thus obtained was considered as representing the carbon dioxide for the middle date. This process was repeated until five periods were obtained for each treatment.

FIGURE 3.—Set-up of experiment in the greenhouse.

EXPERIMENTAL RESULTS

EFFECT OF SOIL TREATMENTS UPON EVOLUTION OF CARBON DIOXIDE IN NORFOLK SAND

The data obtained by the foregoing process are presented in figures 4 to 7. A study of these data shows that the amount of CO_2 formed in the soils to which organic matter was applied exceeded that produced in soils to which no organic matter was applied. The CO_2 evolved from soils treated with legumes exceeded, for the first 27 days, that evolved from soils treated with Natal grass or manure, which is in keeping with the results reported by Waksman and Tenney (*7*) relative to the influence of the nitrogen content of organic matter. The maximum quantity of CO_2 in every instance was evolved at the time the first measurement was made and thereafter diminished until, at the end of 4 months, there was an apparent constant rate of decomposition, and the amount was equal to that formed from the virgin check soil. The rate of decomposition found here is in accord with the results of Martin (*4*) and others.

The evolution of CO_2 in the soils kept in the greenhouse was at a maximum during the first 60 days, whereas in the soils kept under field conditions the maximum amount was formed during the first 24 days. The time required for CO_2 formation to become constant outside the greenhouse was about half that required in the greenhouse. This indicates a quicker action on the part of the organisms outside the greenhouse, although the total action was not so great as under greenhouse conditions. From this it is concluded that the greenhouse

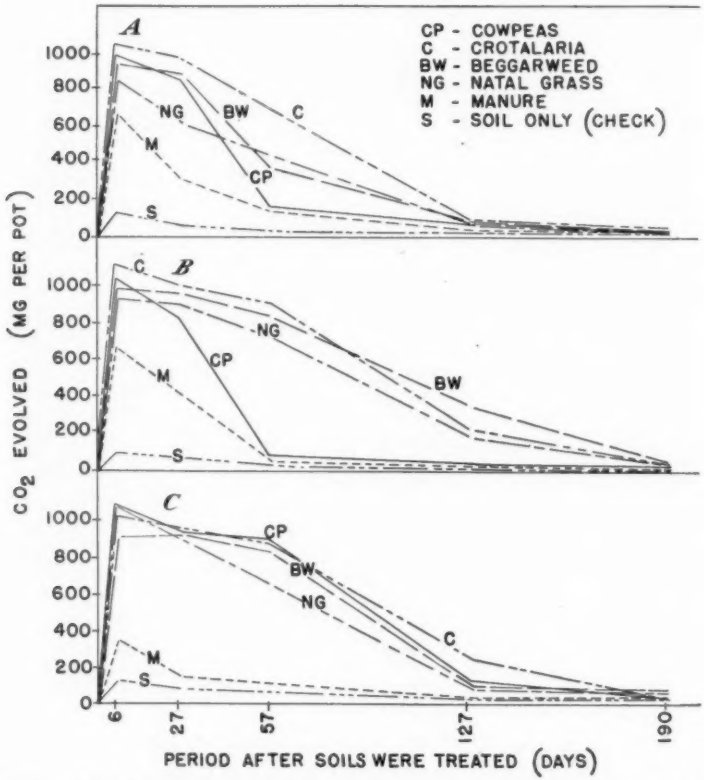

FIGURE 4.—Effect of various soil treatments on the evolution of CO_2 in Norfolk sand, in the greenhouse with seedlings: *A*, indicated materials added alone; *B*, indicated materials and $(NH_4)_2SO_4$ added; *C*, indicated materials and a complete fertilizer added.

conditions were more favorable for complete decomposition than were the field conditions.

Figure 8 represents the total CO_2 collected from the soils under each treatment during the entire period of the experiment. These curves show that generally more CO_2 was evolved from the soils bearing citrus seedlings than from those left fallow and that more was evolved from the soils kept in the greenhouse, where optimum soil moisture was maintained, than from the soils outside the greenhouse. The addition of inorganic fertilizer caused an increased

total CO_2 evolution over that of the organic materials alone from practically all the soils containing seedlings. The complete fertilizer and ammonium sulphate alone appeared to be of approximately equal value in this respect. Whether this increase was due to greater biological activity or to increased root growth, it is difficult to say. As there was a tendency toward a reduction in the evolution of CO_2 from the fallow soils to which ammonium sulphate, in the greenhouse,

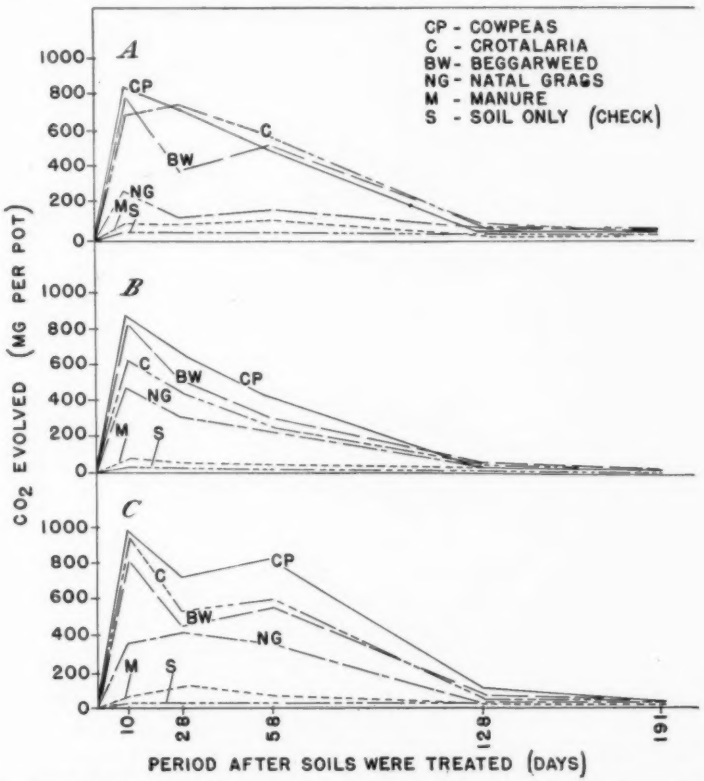

FIGURE 5.—Effect of various soil treatments on the evolution of CO_2 in Norfolk sand, in the greenhouse, without seedlings: *A*, Indicated materials added alone; *B*, indicated materials and $(NH_4)_2SO_4$ added; *C*, indicated materials and a complete fertilizer added.

and complete fertilizer outside the greenhouse, had been added, it might be concluded that the increase of CO_2 from the cultivated soils was due to increased root growth. The inorganic fertilizers must have been responsible for increased root growth, the increased root growth possibly having a corresponding effect on the amount of CO_2 evolved. The addition of inorganic salts had no influence in the soils to which no organic matter had been added.

DETERMINATION OF FREE AMMONIA IN SOIL AIR

It was thought advisable to test for ammonia formation in connection with this work. Therefore, 10 cc of a N/10 solution of hydrochloric acid was placed in the test tube between the earthenware pot and the CO_2 absorption cylinder. This process was followed for a

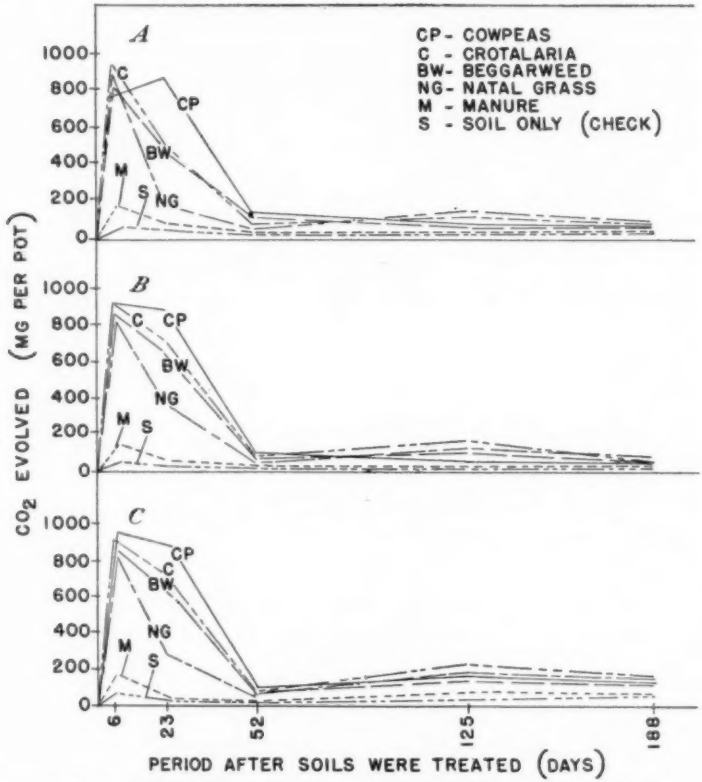

FIGURE 6.—Effect of various soil treatments on the evolution of CO_2 in Norfolk sand, outside the greenhouse, with seedlings: *A*, Indicated materials added alone; *B*, indicated materials and $(NH_4)_2SO_4$ added; *C*, indicated materials and a complete fertilizer added.

period of about 6 months each time the CO_2 was collected. The time covered in this process considerably exceeded that of the greatest activity of the micro-organisms, as measured by the CO_2 evolved.

After the soil air had been passed through the acid solution, the acid was titrated against N/10 sodium hydroxide solution. At no time during this period was there any indication of loss of nitrogen through volatilization of ammonia.

THE EFFECT OF SOIL TREATMENTS UPON THE ACCUMULATION OF NITRATES IN NORFOLK SAND

The best material for improving the physical or chemical properties (or both) of a soil is not necessarily the one that forms the greatest amount of nitrates or other plant food. It is the one that produces nitrates at a rate only sufficient to feed the crop adequately. Any production in excess of this amount is subject to loss through leaching. Whether or not any nitrogen is made available will depend

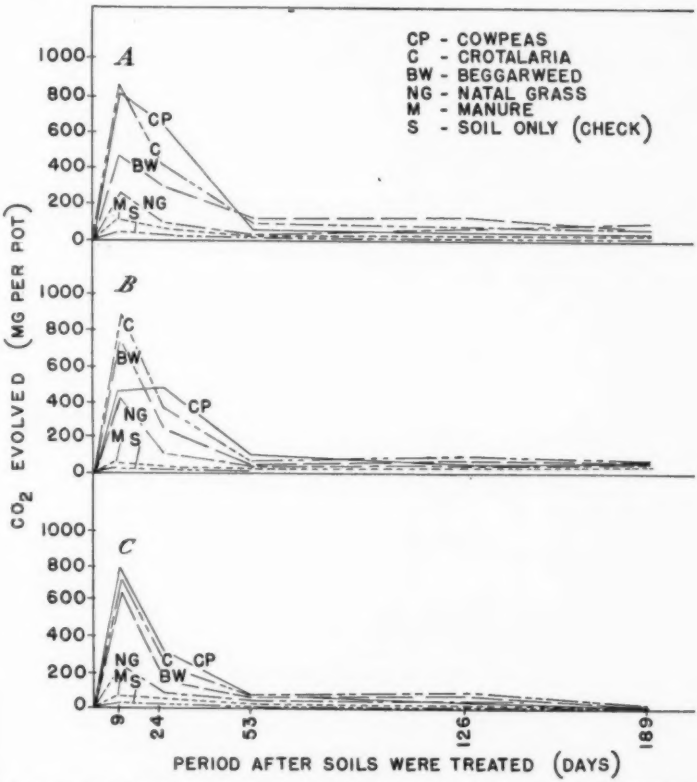

FIGURE 7.—Effect of various soil treatments on the evolution of CO_2 in Norfolk sand, outside the greenhouse, without seedlings: *A*, Indicated materials added alone; *B*, indicated materials and $(NH_4)_2SO_4$ added; *C*, indicated materials and a complete fertilizer added.

in part at least on the amount of nitrogen in the plant material. Likewise the rate of decomposition will depend on the supply of nitrogen available for the organisms.

As the growing citrus seedlings would assimilate some of the nitrates, the results from the fallow pots only are presented. There being no drainage from the pots remaining in the greenhouse because of the control of the moisture content, only the drainage water from the pots kept outside the greenhouse had to be considered. The

nitrate in the drainage water from these pots was determined each time the water was collected. The quantity of nitrate in the water from a given pot was added to the total quantity of nitrate found in the soil from that pot the next time it was sampled subsequent to the date of drainage. By following this method, all the nitrate could be accounted for and comparisons could be made directly with the quantity found in similar pots in the greenhouse.

At definite intervals the soil in each pot was sampled by the use of a suitable borer and taken to the laboratory for analysis. Twenty grams of this soil were weighed into Erlenmeyer flasks, 100 cc of distilled water added, and the mixture was thoroughly shaken, and after standing for a short time, filtered. The procedure from this point was that of Schreiner and Failyer (*5*). As in the case of the carbon dioxide studies, 22 nitrate determinations were made on each

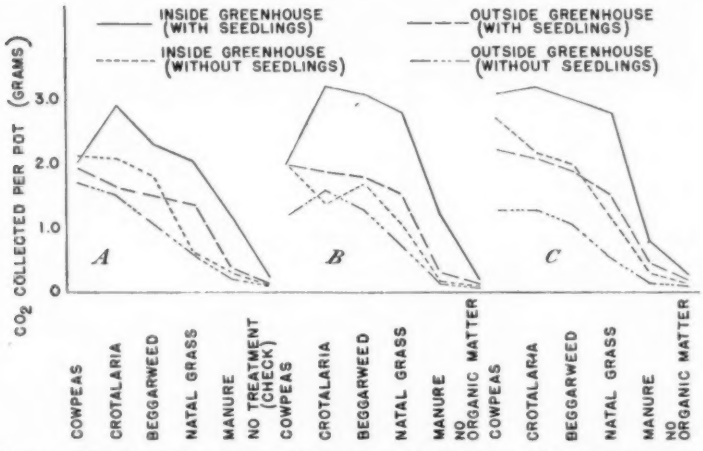

FIGURE 8.—Effect of various soil treatments upon the total CO_2 collected from the soil during the experimental period of 268 days: *A* Indicated materials added alone; *B*, indicated materials and $(NH_4)_2SO_4$ added; *C*, indicated materials and a complete fertilizer added.

soil. The method of averaging the data previously employed was followed in these studies.

It appears that the percentage of total nitrogen in the material which is changed into nitrates might be a better criterion of the value of the material than total nitrate accumulation. The curves presented in figures 9 and 10 have been drawn from data obtained as follows: The amounts of nitrate found at definite intervals, after deducting that found in the respective checks, were divided by the actual weights of nitrogen (calculated as nitrates) added to the soil in the organic matter, or inorganic fertilizer. The curves show the actual percentage of nitrate found in the soil at certain intervals, which was derived only from the organic materials.

The zero (0) line in the figures represents the quantity of nitrate found in the virgin soil. The curves above the zero line represent the percentage of nitrate accumulated from the organic-matter treatments alone, and the curves below the zero line represent nitrate

assimilation. The curves for ammonium sulphate and complete fertilizer show the quantity of nitrate produced from these materials only. The curves bring out the difference in the ability of the various types of organic and inorganic materials to produce nitrates under various conditions.

The maximum nitrate accumulation in the greenhouse and in pots kept under field conditions occurred at different times, and the actual percentage of nitrogen changed to nitrates was different. Not only

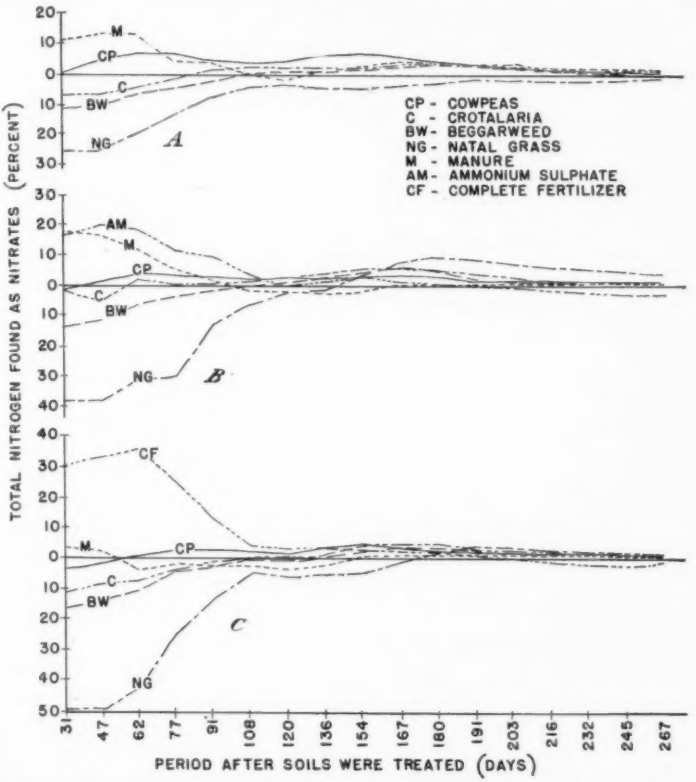

FIGURE 9.—Effect of various soil treatments on the formation of nitrates in the soil, outside the greenhouse, without seedlings: *A*, Indicated materials added alone; *B*, indicated materials and $(NH_4)_2SO_4$ added; *C*, indicated materials and a complete fertilizer added.

was the period of greatest nitrate accumulation more prolonged in the soils in the greenhouse, but the percentage of nitrogen transformed was also greater. The maximum transformation from all the organic materials in the soils kept outside the greenhouse occurred after the end of 3 months, while for the same treatments in the greenhouse maximum transformation occurred much earlier.

When the individual materials which were kept under field conditions are considered (figs. 9, 10) it is found that the rate at which nitrates were formed from nitrogen in the various materials, in the

order of decreasing percentage, was as follows: Complete fertilizer, ammonium sulphate, manure, cowpeas, crotalaria, beggarweed, and Natal grass, regardless of any additional treatment. This is what

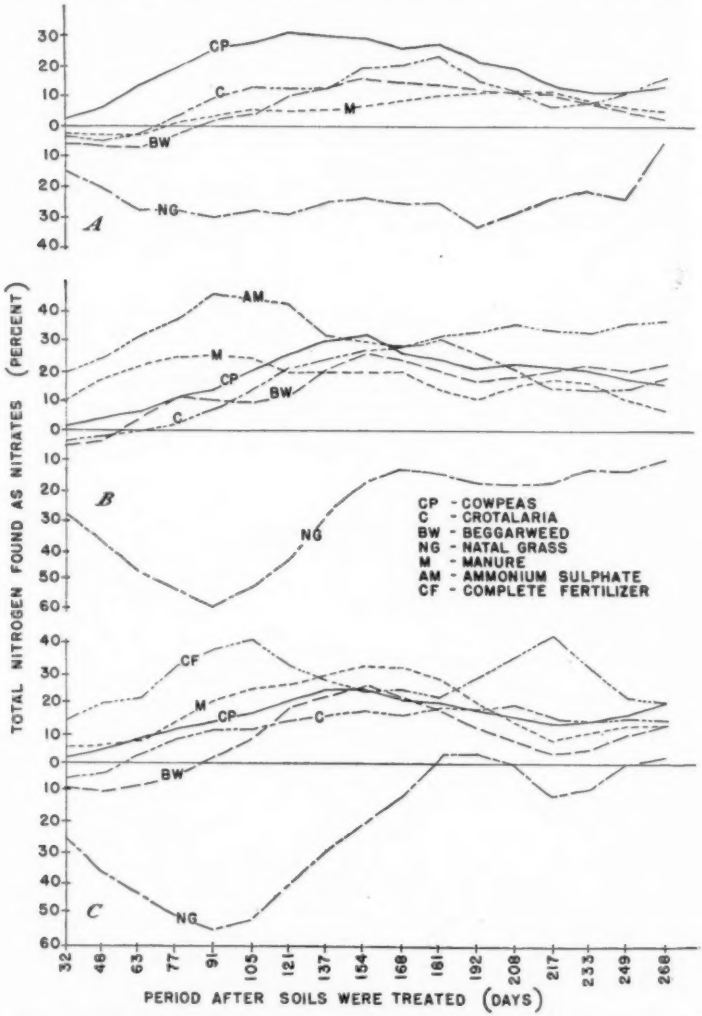

FIGURE 10.—Effect of various soil treatments on the formation of nitrates in the soil, in the greenhouse, without seedings: *A*, Indicated materials added alone; *B*, indicated materials and $(NH_4)_2SO_4$ added; *C*, indicated materials and a complete fertilizer added.

would be expected from the nature of the materials. The only significant difference in the rate of nitrate accumulation from the organic materials, as brought about by the addition of inorganic salts, was in the case of Natal grass. This, too, would be expected since the

organisms would naturally utilize the nitrogen in inorganic fertilizers first, as it occurred there in a more available form. This effect on Natal grass was also observed under greenhouse conditions.

The curves representing the data secured under greenhouse conditions (fig. 10) indicate that the nitrogen in the complete fertilizer and that in ammonium sulphate were changed to nitrates at practically the same rate and that the nitrogen in these materials was converted more rapidly than that in any of the organic materials. In rapidity of oxidation of nitrogen the organic materials ranked as follows: Cowpeas, manure or cowpeas treated with the inorganic salts, crotalaria, beggarweed, manure alone, and Natal grass.

Apparently some combination of Natal grass and a nitrogenous inorganic material, such as ammonium sulphate, should be a satisfactory combination of materials to add to the soil, for this combination showed the smallest percentage oxidation of the nitrogen from the organic material.

SUMMARY AND CONCLUSIONS

A study was made of the comparative rate of decomposition of various organic materials in Norfolk sand and of the effect of the addition of inorganic fertilizers on the rate of decomposition of these materials.

The amount of CO_2 evolved and the nitrates accumulated were used to measure the rate of decomposition of organic matter. The CO_2 evolved from the soils treated with legumes exceeded for the first 27 days the CO_2 evolved from the soil treated with Natal grass or manure. In every instance the maximum quantity of CO_2 was evolved at the beginning of the experiment and gradually diminished thereafter. There was an apparent constant rate of decomposition, as measured by CO_2 evolution, at the end of about 4 months, when the amount formed in the treated soil was approximately equal to that formed in the virgin soil. The period of maximum CO_2 evolution in the case of the soils kept in the greenhouse occurred during the first 2 months of the experiment, whereas that for soils kept under field conditions was formed during the first 30 days.

More CO_2 was evolved from the soils bearing citrus seedlings than from those kept fallow, and the greatest quantity of CO_2 was produced from the soil kept in the greenhouse. The addition of inorganic nitrogenous fertilizers increased the amount of CO_2 given off from all soils bearing citrus seedlings but this increase was possibly due to the increased root growth.

There was no evidence of any loss of nitrogen from these soils through volatilization of ammonia.

In the soils treated with Natal grass there appeared to be considerable nitrogen assimilation. A striking difference in the availability of the nitrogen in the organic materials under different conditions was noted. A greater percentage of the nitrogen in the organic materials was made available under greenhouse conditions, and this greater availability was distributed throughout the period of the experiment.

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THE EFFECT OF GRINDING ON THE DIGESTIBILITY OF CORN BY PIGS AND ON ITS CONTENT OF METABOLIZABLE ENERGY [1]

By W. P. GARRIGUS, *graduate student*, and H. H. MITCHELL, *chief in animal nutrition, Department of Animal Husbandry, Illinois Agricultural Experiment Station*

INTRODUCTION

The effect of grinding on the value of corn (*Zea mays*) as a feed for pigs has been studied mainly in feeding experiments, the results being measured by the rapidity and feed economy of the gains secured. In the United States it has in general been found, according to Morrison,[2] that for young pigs ground corn is not appreciably superior to whole corn, but for older pigs, variable percentage advantages in economy of gains may result from the feeding of ground corn. However, a more recent summary by Crampton [3] indicates no advantage due to grinding at any weight of pig. In sharp contrast to the American experiments are the results of Hansson [4] in Sweden, who calculated from his own feeding experiments on pigs that the productive value of maize was increased by fine grinding by as much as 15 to 18 percent.

Such findings as are obtained in feeding experiments are the resultants of a number of factors, including effects of grinding on the digestibility of the corn, on its palatability, and, as Crampton points out, on its wastage at the feed trough: "Shelled corn lost from the feeder is salvaged by the hogs but ground feed is not * * * Feed rooted out of the trough and lost to the pig is, of course, still charged to the gains made." A complete understanding of the problem, therefore, demands a consideration of each of these factors.

PREVIOUS INVESTIGATIONS

The factor most amenable to quantitative study, and perhaps the most fundamental factor of all, is the effect of grinding corn on its digestibility. However, very little study seems to have been made on this point. A summary of such work as has been found in the literature is embodied in table 1.

The corn in Jordan's experiment [5] was a flint variety. The experimental pig weighed about 50 pounds and received in each of the two digestion trials 787 g of corn daily. The collection period was of 5 days duration.

[1] Received for publication Feb. 18, 1935; issued July 1935.

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In Haberhauffe's experiment,[6] the degrees of fineness of the ground corn studied can be roughly characterized by the percentages passing through a 1-mm round-hole sieve, i. e., 42, 63, and 98. The two pigs weighed initially 87.5 kg. They received daily 4 kg of the corn mixed with water, except that the whole corn was fed mixed with the coarsely ground corn in even proportions. The digestibility of the whole corn was then computed indirectly. Apparently the pigs were not fed or confined separately during the collection period of 7 days.

TABLE 1.—*Previous investigations of the effects of grinding on the digestibility of corn when consumed by swine*

Condition of corn	Number of pigs	Digestion of coefficients obtained					Authority and reference
		Organic matter	Crude protein	Crude fat	Crude fiber	N-free extract	
Whole kernels	1	83.4	68.7	45.6	38.3	88.8	Jordan.[1]
Finely ground	1	91.2	86.1	81.7	29.4	94.2	
Whole kernels	2	89.6	78.6	51.7	52.4	94.7	Haberhauffe.[2]
Coarsely ground	2	90.4	79.2	59.7	55.7	95.0	
Medium ground	2	93.7	82.4	79.0	75.9	96.8	
Finely ground	2	94.9	87.2	78.7	78.0	97.5	

[1] See footnote 5. [2] See footnote 6.

The experiment of Jordan [7] in particular indicates a marked improvement in the digestibility of corn by grinding it to a fine meal, possibly because a flint variety of corn was used. In Jordan's study, grinding improved the digestibility of the organic matter 9.4 percent, that of protein 25.3 percent, and that of nitrogen-free extract 6.1 percent. The results obtained by Haberhauffe indicate less improvement for the finely ground meal, i. e., 5.9 percent in the digestibility of the organic matter, 10.9 percent in the digestibility of the protein, and 3.0 percent in the digestibility of the nitrogen-free extract. The meals of intermediate fineness show an intermediate but graded improvement.

It appears from these two experiments, constituting the only published work on the problem, that the effect of grinding on the digestibility of corn has not yet been precisely measured. Since the nutritive advantages of the grinding of corn must be balanced against the cost of grinding in deciding whether and when this method of preparing corn is economical, the need of more work of this character seems evident. The experiment reported below is a further contribution to this problem.

PLAN OF EXPERIMENT

The subjects of the experiment were 5 Chester White barrows and 1 Hampshire barrow (pig 4), weighing initially from 135 to 196 pounds. Each of the pigs received daily either 1,300 g or 1,500 g of corn, depending upon appetite, with no other feed. The digestibility and metabolizable energy content of the whole corn and of the same corn after grinding was determined with 5 of the 6 pigs, 1

[6] HABERHAUFFE, W. ÜBER DEN EINFLUSS DER ZUBEREITUNG AUF DIE VERDAULICHKEIT DER FUTTERMITTEL. Jour. Landw. 74: [191]–230. 1926.
[7] JORDAN, W. H. See footnote 5.

pig refusing to consume the whole corn in adequate amounts after a feeding period on ground corn. Three of the pigs received the whole corn first, and three received the ground corn first. In an immediately following period, the rations were reversed, each pig continuing, however, on the same amount of feed.

Each feeding period consisted of from 14 to 17 days, during the last 10 days of which both feces and urine were collected. The animals were confined throughout in metabolism crates very similar in size and design to the crate described by Forbes .[8] The feces of the collection periods were separated from those of adjacent periods by giving ferric oxide (equal in weight to 3 percent of the morning feed) to the pigs on the morning of the first day of the collection and again on the morning of the day following the termination of the period. The feces were dried at a low temperature and analyzed for moisture, nitrogen, crude fiber, and gross energy (heat of combustion), while the urines (with washings) were analyzed for nitrogen and gross energy.

TABLE 2.—*Chemical composition and gross energy value of the corn samples*

Sample of corn	Dry matter	Total nitrogen	Crude fiber	Gross energy	Sample of corn	Dry matter	Total nitrogen	Crude fiber	Gross energy
	Percent	*Percent*	*Percent*	*Calories per gram*		*Percent*	*Percent*	*Percent*	*Calories per gram*
Whole 1	88.73	1.67	3.80	3.967	Whole 2	89.50	1.66	3.58	4.025
Ground 1	88.99	1.67	3.89	4.033	Ground 2	88.90	1.67	3.70	4.015

The analysis of the corn samples, collected daily at feeding time for the two series of 10-day collection periods, is shown in table 2. The fineness of grinding was determined by passing portions of the ground corn through sieves of three sizes, 12 mesh (2.1 mm), 20 mesh (1.3 mm), and 40 mesh (0.64 mm). Ninety-five percent of the corn passed through the coarsest sieve, 58 percent through the intermediate sieve, and 29 percent through the finest sieve. The fineness of grinding appears to approximate the "medium ground" sample of Haberhauffe [9] (table 1).

RESULTS OF THE EXPERIMENT

While the pigs were on the whole corn ration an attempt was made to determine what percentage of the consumed kernels appeared unbroken in the feces. By counting a 600-g portion of the corn, it was found to contain 350 kernels per 100 g. For each pig an aliquot of one-fifth of the unground feces for the collection period was carefully inspected and the unbroken kernels were separated and counted. The percentages (by number) of the consumed kernels thus recovered were 0.38 for pig 1, 1.10 for pig 3, 1.10 for pig 4, 0.38 for pig 5, and 37.90 for pig 6. The outstanding position occupied by the last animal is noteworthy, and was probably the result of defective teeth, although the truth of this supposition was not confirmed.

[8] FORBES, E. B. A METABOLISM CRATE FOR SWINE. Ohio Agr. Expt. Sta. Circ. 152, pp. 75-85, illus. 1915.
[9] HABERHAUFFE, W. See footnote 6.

TABLE 3.—*Results of the digestion and the metabolism trials with whole and ground corn when consumed by swine*

Pig no.	Body weight	Condition of corn	Daily intake in food				Coefficients of digestion				Metabolizable energy		Nitrogen balance per day
			Corn	Dry matter	Gross energy	Nitrogen	Dry matter	Gross energy	Nitrogen	Crude fiber	Total	Per kilogram of dry matter consumed	
	Kilograms		*Grams*	*Grams*	*Calories*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Calories*	*Calories*	*Grams*
1	83.5	Whole	1,500	1,342	6,037	25.05	85.1	82.5	65.0	68.5	4,847	3,612	+3.19
	78.9	Ground	1,500	1,335	6,049	25.05	87.4	85.9	77.2	70.5	5,010	3,753	+4.20
2		Whole											
	78.5	Ground	1,500	1,335	6,049	25.05	89.2	88.0	83.2	73.6	5,153	3,860	+5.34
3	62.1	Whole	1,300	1,153	5,157	21.71	86.0	83.6	63.0	77.0	4,174	3,620	+3.32
	63.5	Ground	1,300	1,156	5,219	21.71	87.6	86.3	80.0	72.9	4,367	3,778	+3.32
4	67.1	Whole	1,300	1,163	5,232	21.71	88.4	86.6	73.1	70.0	4,407	3,789	+4.01
	64.4	Ground	1,300	1,157	5,243	21.71	88.8	87.2	76.2	73.1	4,422	3,822	+4.28
5	83.9	Whole	1,500	1,331	5,950	25.05	91.4	89.6	80.0	(1)	5,124	3,850	+4.31
	86.2	Ground	1,500	1,333	6,022	25.05	90.4	89.0	85.1	75.7	5,125	3,845	+4.11
6	89.4	Whole	1,500	1,331	5,950	25.05	82.0	80.0	71.0	46.5	4,575	3,437	+2.76
	90.7	Ground	1,500	1,333	6,022	25.05	87.9	86.1	78.2	70.9	5,008	3,757	+4.05

[1] This sample of feces was accidentally thrown out before the crude fiber determination was made.

The most significant results of the digestion and metabolism trials are assembled in table 3. It is evident from the coefficients of digestibility that, except for protein (nitrogen), the constituents of the ground corn were not digested to a markedly greater extent than the constituents of the unground corn. Omitting the incomplete results for pig 2, the average coefficients of digestibility of the whole corn and ground corn were, respectively, 86.6 and 88.4 for dry matter, 84.5 and 86.9 for gross energy, and 70.4 and 79.7 for protein. The percentage improvement in the digestibilities were 2.1 for dry matter, 2.8 for gross energy, and 13.2 for protein. For the four pigs for which the coefficient of digestibility of crude fiber was determined, the averages were 65.5 for the whole corn and 71.8 for the ground corn, but if the atypical results of pig 6 are omitted, the averages are very nearly the same, i. e., 71.8 and 72.2.

It is especially noteworthy that pig 6, which appeared to be passing unbroken and undigested more than a third of all whole corn kernels consumed, digested the whole corn fairly well, except for the crude fiber. While grinding improved the digestibility of corn for this pig somewhat more than for the other pigs, the relative improvement was far less than would be expected from the physical examination of the feces for unbroken kernels, proving the latter method to be quite unreliable as a criterion of completeness of digestion. The apparently unbroken kernels must have been very largely denuded of their digestible contents by the digestive enzymes.

Since corn is preeminently an energy food, the metabolizable energy values of whole and ground corn are of great practical significance. But here also the improvement brought about by grinding is slight, except for pig 6. In fact, for one pig (no. 5) no improvement at all resulted. For all five pigs upon which complete data were obtained, the metabolizable energy per kilogram of dry matter averaged 3,662 calories for the whole corn and 3,791 calories for the ground corn, representing an improvement of only 3.5 percent.

The pigs were in positive nitrogen balance in all periods. The consistently greater digestibility of the protein of the ground corn did not, however, promote consistently higher nitrogen balances, since without exception the urine from pigs on the ground corn ration contained more nitrogen than that from the same pigs on the whole corn ration. In one pig (no. 5) the lesser wastage of nitrogen in digestion on the ground corn ration was more than offset by the greater wastage in metabolism, while for another pig (no. 3) these two tendencies just balanced each other. Thus, the 13-percent improvement in the digestion of the nitrogen of the corn brought about by grinding was largely lost in metabolism, a result suggesting that the digestible nitrogen escaping digestion in the whole corn represents a fraction of the corn protein that is of low value biologically.

SUMMARY AND CONCLUSIONS

The relative digestibility of whole and ground corn and their content of metabolizable energy were determined on five pigs weighing from 135 to 196 pounds.

Grinding corn to a medium degree of fineness (29 percent passing a 40-mesh sieve, 58 percent a 20-mesh sieve, and 95 percent a 12-mesh sieve) increased the digestibility of the protein by 13 percent, but the digestibility of the gross energy of the corn was raised only 2.8 percent. The metabolizable energy was also only slightly improved; i. e., 3.5 percent. Furthermore, the appreciable advantage in protein digestibility occasioned by the grinding of corn was largely lost by greater losses of nitrogen incurred in metabolism, so that the net effect on the nitrogen balance of an animal receiving an exclusive ration of corn was slight and inconstant.

The net effect of grinding upon the nutritive value of corn for pigs of the weights used in this test is to increase its value as a source of energy by 3.5 percent. Its value as a source of protein was not appreciably altered.

STUDIES ON BEAN RUST CAUSED BY UROMYCES PHASEOLI TYPICA[1]

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INTRODUCTION

Bean (*Phaseolus vulgaris* L.) rust, caused by *Uromyces phaseoli typica* Arthur, occurs in widely separated regions of the United States and has been reported from Europe, continental Asia, Japan, Australia, and other parts of the world. The occurrence of the disease in various parts of the United States is extremely sporadic as regards both regions and years. Bean rust occurs to some extent almost every year in certain States along the Atlantic seaboard and in many States in the South. In the Southeastern States and in some of the Northern States, for example, in New York and Michigan, it usually appears in the late summer or early fall after the bean crop is about matured and when the danger from severe damage is over. In Florida, on the other hand, epidemics have occurred in the late winter or early spring in such severe form that the plants were defoliated and the crop was practically ruined.

A study of the California, Idaho, and Colorado areas with respect to bean rust reveals the interesting fact that some very striking climatological and ecological differences exist. Although the climatic conditions of Colorado and Idaho seem to be quite similar, there are differences sufficient to suggest causes for the occurrence of rust epidemics in the one and not in the other State. If California is included in the comparison, there are three areas that differ in regard to the occurrence of rust—one in which epidemics occur yearly, one in which they occur occasionally, and one in which rust is never or rarely observed.

Climatological data have been collected for 3 years in the three Western States just mentioned. These data, while perhaps not explaining fully the potentialities of the different areas with respect to rust epidemics, may contribute much to an explanation of their occurrence.

In addition to observations on the epidemiology of the fungus, studies have been conducted regarding its various spore forms, their longevity and germination; the influence of temperature, humidity, and light on infection; the time required for infection; the correlation of weather conditions with rust occurrence and epidemics; physiologic forms; varietal susceptibility and resistance; and control and dissemination. This paper summarizes the results of researches carried on for about 5 years in the field and laboratory, particularly regarding phases of the problem not previously investigated or only incompletely investigated by other workers.

[1] Received for publication Dec. 26, 1934; issued July 1935.

[2] The writers are indebted to I. C. Jagger, senior pathologist of this Division, who tested the susceptibility and resistance to rust of many of the bean varieties grown at Chula Vista, Calif., over a period of 2 years.

SOURCES OF MATERIAL

Bean rust material has been collected or received from California, Colorado, Virginia, Florida, South Carolina, Tennessee, Mexico, and Australia. Spores from the various sources, except those obtained from Australia, were virulent and gave typical symptoms of the disease when used for inoculation. The collections from different areas were compared to ascertain whether there were any distinct biological or morphological forms.

EXPERIMENTAL METHODS

In studying the various phases of bean rust, such as the relation of temperature and humidity to infection, length of time required for infection, spore germination, and other phenomena, rust material from a single source was employed.

Abundant spore material was maintained by culturing the rusts on the Pinto variety in the greenhouse. This variety is a very susceptible field bean and grows well under greenhouse conditions. It was used almost entirely except where variety tests were being conducted or where a differential host was desired. The Tennessee Green Pod, another very susceptible variety, was used in a few experiments.

In order to have at hand an abundance of rust material, plants were inoculated about every 7 to 10 days by atomizing them with urediospores suspended in water or by spreading them on the leaf with a camel's-hair brush. The plants were then confined in an infection chamber held nearly at saturation and at a temperature of about 18° to 20° C. for 15 to 24 hours. The temperature varied somewhat with the season, but the best results were obtained when it was not above 21°. The minute, pale-colored flecks beneath the epidermis were the first evidence of infection under greenhouse conditions and appeared in about 4 or 5 days. At the end of about 10 days the pustules opened.

SPORE FORMS

LIFE CYCLE

The life cycle of the bean rust in the greenhouse calls for particular mention. Andrus (*1*)[3] has previously reported on observations of the aecial and spermogonial stages in the development of the bean and cowpea rust. *Uromyces phaseoli typica* forms teliospores abundantly in the fall on beans in Colorado, and material from there as well as from California has been employed for most of the experimental work. If field-collected teliospores are germinated and then placed on bean leaves in the greenhouse and given suitable temperature and humidity conditions, fertile aecia are formed, followed after reinfection by the production of uredia and teliosori. On the other hand, a limited number of tests have indicated that if teliospores produced in the greenhouse are germinated and placed on the leaves under conditions similar to those just described, only sterile aecia are produced, even though the nectar from different aecia is mixed. Successful infection with greenhouse-produced teliospores was ob-

[3] Reference is made by number (italic) to Literature Cited, p. 758.

tained on only one occasion. In this case the several aecial infections all proved to be of the same sex and remained sterile after transfer of spermatia. Because of this fact and the limited number of tests made, it cannot be said that the complete life cycle of the bean rust has been reproduced under greenhouse conditions. It would seem that if a beginning is made with teliospores of the proper origin the full life cycle of the bean rust is possible under greenhouse conditions.

TELIOSPORE FORMATION

Observations have been made on teliospore production under greenhouse conditions and on the variation in the ability of cultures of different origin to produce teliospores. Bean rust has been collected from various parts of the United States, and three areas—southern California, Colorado, and Virginia—may be selected to illustrate the differences in teliospore formation. In both Colorado and Virginia teliospores develop abundantly in the latter part of the summer before the vines have been killed by frost. On the other hand, they rarely form in southern California, although the vines are killed by frost during the winter months. In an abundance of material from there, only 2 or 3 pustules containing teliospores have been found; the strain seems to have almost lost the power to form teliospores. Out of many inoculations made in the greenhouse with spores from California, teliospores have never developed, while the production of such spores on plants inoculated with material from Colorado or Virginia is of common occurrence.

In tropical and subtropical regions the preponderance of uredia of different rust species has frequently been noticed and has sometimes been attributed to the lack of recurring conditions that retard growth. Many theories have been advocated to explain this phenomenon. Ivanov (*15*) found that cool temperatures checked urediospore production and increased the development of teliospores, while Smith (*28*) concluded that abundance of soil moisture stimulated the development of the host and increased its resistance, as a result of which the fungus (*Puccinia asparagi* DC.) was retarded and forced to develop telia. These results are contrary to those obtained by Sheldon (*26*, *27*), Stakman and Levine (*29*), Ward (*31*), Arthur (*2*), and others, who maintain that, as a rule, whatever favors the development of the host also favors the rust. In 1928 Waters (*32*) carried out an elaborate series of experiments designed to study the influence of environmental factors on the production of teliospores and urediospores on a number of hosts, including beans, and concluded that all rusts studied are directly dependent upon photosynthetic activity of the host. He states (*32*, *p. 209*):

> Any single factor or set of factors, such as light, temperature, and moisture, or, as in the case of climate, a complex of these factors may so influence and do influence the metabolism of the host, that the fungus reacts by changing from the uredinial to the telial generation, or, under proper manipulation, in the reverse direction.

To the writers the explanation quoted above does not seem entirely adequate. Bean rusts from various sources have been propagated in the greenhouse for about 5 years and under a considerable diversity of conditions. Those originating in Colorado and Virginia, where teliospores normally develop, produced them abundantly in the greenhouse. On the other hand, the strain from California has

consistently failed to produce teliospores under any of the conditions to which it has been exposed. One of the usual concepts regarding the behavior of rusts is that teliospore formation is more or less coincident with the aging of the plant and the mobilization of food materials for the production of spores (*3*). Measured by these standards, the bean rust should develop teliospores in California and in the greenhouse as well as do the rusts from other sources. In a consideration of these rusts under various conditions one cannot ascribe to environment alone the difference in their behavior in respect to teliospore formation.

Gassner (*12*) observed differences in the time of teliospore formation among several species of *Puccinia* on cereals. Withholding mineral nutrients from the host and mutilating the host tissue failed to hasten the production of teliospores.

Raines (*25*) suggested that the protoplasmic constitution of the fungus may be a factor in teliospore production. In experiments conducted by him he was able to show a variation on the part of *Puccinia coronata* Cda. (referred to by him as *P. coronifera* Kleb.) in the production of teliospores. By selecting spores from pustules containing no teliospores he was able to decrease the number of teliospores from pustules; likewise by selecting spores from pustules containing teliospores he increased the number of such pustules. In view of these results, it is conceivable that a strain of rust might be developed which had entirely lost its power to form teliospores. It is not improbable that the rust in southern California, having been propagated naturally for a number of generations from the urediospores, has lost its ability to produce teliospores. The winter season is short, so the urediospores would have to survive only a few weeks to carry the rust from one crop season to the next.

In an attempt to stimulate teliospore production, the methods employed by Waters (*32*) were tried. From the results obtained by the writers there are reasons to believe that there are at least two forms of bean rust that differ genetically in the ability to form teliospores at some stage of their development. Within the teliospore-producing strain the abundance of teliospores as well as the time when they appear may be controlled to an appreciable extent by altering host metabolism.

LONGEVITY OF UREDIOSPORES AND TELIOSPORES

A study of the longevity of rust spores has shown that the urediospores of some rusts will retain their viability for several months and in some cases for a year, and the teliospores for a longer time. The evidence contributed by many investigators indicates that the viability of the spores is closely correlated with the temperature and humidity at which they are stored. In this connection it is interesting to note the results of Peltier (*23*), who found that the urediospores of *Puccinia graminis tritici* Eriks. and Henn., form III, when stored at humidities varying from 38 to 70 percent and at temperatures of 5° to 15° C., retained their viability better than when stored at higher temperatures. The viability was reduced appreciably when the spores were stored at high and low humidities. On the other hand Bailey (*4*) found a humidity of 20 to 40 percent at a temperature of 23° to be the optimum for the storage of urediospores of *P. helianthi* Schw., which retained their viability for 185 days under such condi-

tions. Hart (*14*) found storage of *Melampsora lini* (Pers.) Lév. spores at a relative humidity of 40 percent and a temperature of 7° to be the most favorable for longevity. Lambert (*19*) showed that the teliospores of wheat rust usually lose their viability a few months after maturity, but if kept in cold storage their longevity may be prolonged for nearly a year.

The investigations conducted by the writers on the longevity of bean rust spores are not intended to be complete. They were designed primarily to determine whether the spores were capable of maintaining their viability for a considerable length of time under what might be regarded as favorable conditions, and not what effect different temperature and humidity conditions would have on their longevity.

In October 1931, bean leaves with abundant rust pustules containing both urediospores and teliospores were collected and stored in an incubator at 9° C. with the relative humidity at approximately 73 percent. This experiment was intended to show the longevity of urediospores under known conditions and not the longevity of the teliospores, which were previously shown to germinate for a number of months after maturity. However, teliospores were later germinated on drops of water on cover slips in Petri dishes at intervals of 2 weeks. This method was found suitable for the purpose. Each sample was kept under observation for about 3 weeks. From 50 to 60 percent of the teliospores had germinated at the end of 207 days, after which no more tests were made.

The longevity and viability of the urediospores were determined by a somewhat different method. Instead of testing spore germination in water on slides, the spores were used to inoculate bean plants in the greenhouse. After an initial storage period of 10 weeks, young plants of the Pinto variety were subjected to infection at intervals of 2 weeks, with the result that a few scattered infections were obtained at the end of 26 weeks, but none after 28 weeks. These results indicate that bean rust might be carried over from one season to the next by means of the urediospores.

COMPARISON OF BEAN, COWPEA, AND STROPHOSTYLES RUSTS

The bean rust and the cowpea (*Vigna sinensis* (L.) Endl.) rust were at one time thought to be identical. Fromme (*7*) later proved them to be distinct pathogenetically; he also proved that there were morphological differences, from which he concluded that the cowpea rust was a clearly distinct species and assigned it to *Uromyces vignae* Barcl. A new description of the species was appended. The work of Fromme was so complete as to leave little doubt of the correctness of his conclusions. In view of that fact the discussion of the cowpea rust will be confined to some additional evidence acquired in connection with the investigations of bean rust.

In 1929 badly rusted Blackeye cowpeas were found growing among severely rusted beans in California, which suggested that the two rusts might be identical. A series of cross inoculations were made in which it was found that bean rust collected from widely separated regions would not infect cowpeas and that cowpea rust would not infect beans. Other differences were also noticed. The teliospores of cowpea rust germinate readily soon after maturity, whereas the bean rust teliospores require a rest period or germinate only sparingly

and feebly when first matured. The aecia of both have been produced in the greenhouse. Aecia of bean rust are white; those of cowpea rust are yellow. Likewise the nectar of the bean rust spermogonia is white, and that of the cowpea rust spermogonia is yellow. Morphological differences in the teliospores pointed out by Fromme were also found.

A rust with some points of resemblance to both bean and cowpea rusts was found on *Strophostyles helvola* (L.) Britt. in Virginia, not far from Washington, D. C., which might be referred either to *Uromyces phaseoli typica* or to *U. phaseoli vignae* (Barcl.) Arthur. Teliospores in considerable abundance were collected, and attempts were made to infect beans and cowpeas, but without success. Conversely, *S. helvola* could not be infected with rust from beans or cowpeas. It was difficult to propagate *S. helvola* in the greenhouse, and only a few plants were available for inoculation. In view of the limited number of inoculations, the results are to be interpreted as merely suggestive. Cowpeas and beans, however, were available in abundance for inoculation with the *Strophostyles* rust. Aecia of this rust were developed on *Strophostyles* in the greenhouse by inoculation with sporidia from teliospores. The nectar and the aecia are yellow, and in this respect *Strophostyles* rust resembles the cowpea rust. On the other hand, *Strophostyles* rust resembles bean rust in that a considerable rest period is required before the teliospores germinate readily. *S. helvola* was not readily infected in the greenhouse with rust spores from *Strophostyles* collected in the field. The aecia were formed, but no other form of rust developed. On the other hand, the cowpea and bean rusts produce all spore forms under greenhouse conditions. The writers are inclined to agree with Fromme that *Strophostyles* rust probably represents a different species from that found on either beans or cowpeas.

ENVIRONMENTAL FACTORS

RELATION OF TEMPERATURE TO SPORE GERMINATION

The urediospore seems to be the principal spore form involved in the dissemination of the bean rust and in the damage to the crop that follows. Bean rust occurs at different seasons of the year in regions differing considerably in temperature and subject to wide temperature fluctuations, which suggests that there is some correlation between these factors and the presence of rust and rust epidemics. Temperature alone, of course, is not the limiting factor for infection, since there is also a minimum of atmospheric moisture that will permit spore germination. In case a high humidity persists for only a few hours during a day, it is highly essential that the spores germinate in the briefest time possible if infection is to be established. With this thought in mind a series of experiments was designed to determine the time required for spore germination, the minimum moisture requirement, the optimum temperature, and the temperature range.

The urediospores of the bean rust will germinate as soon as they are mature. It has been noted that they may deteriorate in a short time after maturity and that better infection may be obtained if the spores are taken from freshly opened pustules than if collected from those several weeks old. On the other hand, if they are properly manipulated their virulence may be maintained for a considerable

length of time, as shown (p. 741). The germination experiments were made with spores 1 to 2 weeks old taken directly from the pustules on the leaves. They were sprinkled thinly on a 2-percent potato-dextrose agar in Petri dishes and placed in incubators with temperatures ranging from 0° to 36° C.

The inability to duplicate in the laboratory the conditions that surround the spores in nature has frequently aroused considerable controversy about the interpretation of the results. The germination of the spores under artificial conditions cannot be interpreted as representing exactly what takes place under natural conditions. With this fact in mind, germination tests with bean rust spores were made with the hope that some data might be obtained that would help to explain the occurrence of bean rust in regions where it does not appear annually in an epidemic form.

Figure 1 illustrates the germination of urediospores during a period of 24 hours in controlled temperature chambers. In a consideration

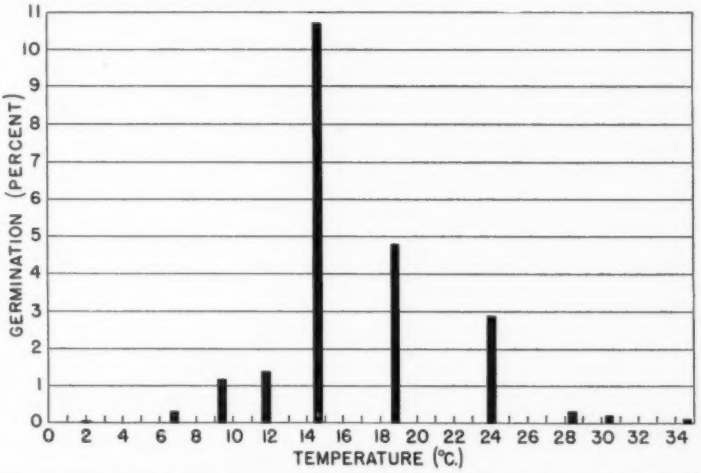

FIGURE 1.—Approximate hourly average percentage of urediospores of *Uromyces phaseoli typica* germinating during a period of 24 hours at constant temperatures.

of these results it must be kept in mind that the optimum temperature for spore germination was considered to be that at which the largest percentage of spores germinated in a fixed period, in this case 24 hours. The germ tubes were longer on an average at the temperatures where the largest percentage of germination took place.

The results show that the percentage of spore germination was not large at any temperature. The percentages of germination were arrived at by the following method: The plates were examined every hour and a count was made of the total number of spores and number of spores that germinated in five different fields under the microscope and averaged. The counts in many cases amounted to several hundred spores. At temperatures of 12°, 15°, and 17° C., spore germination was evident in 2 hours and increased with the length of time. In 4 hours germination had taken place over a range of temperatures from 10° to 31°, and in 5 hours from 7° to 35°.

From figure 1 it is seen that the optimum temperature for the germination of the urediospores, using the percentage of germination at the end of a given time as a criterion, is approximately 14.5° C. Above that optimum the percentage of spore germination decreases rapidly. The minimum was found to be 1.8° and the maximum slightly above 34.5°.

RELATION OF TEMPERATURE TO INFECTION

No attempt was made to determine exactly the maximum and minimum temperatures for infection. The optimum was found to be approximately 17.0° C., which is somewhat higher than the optimum for spore germination. Above and below that temperature the percentage of infection decreased. At 27° no infection was obtained. In studying the lower range for infection, difficulties were encountered because the plants exhibited considerable injury from chilling. The few results obtained indicate that infection would probably take place at a temperature as low as the plant could endure without injury.

RELATION OF HUMIDITY TO INFECTION

The rapidity with which the spores will germinate and infect the host is of primary importance, especially in arid regions where the relative humidity is high enough for spore germination to take place for only a short time during any 24-hour period, which is usually at night. Frequently the humidity is high for a day or two during rainy periods, which rarely occur in the arid regions of the West. The probability is that, in order to cause infection in a dry climate, a rust spore must germinate and infect the host within a few hours.

The results of experiments on the relation of humidity to infection by the bean rust show that within the range of temperatures permitting spore germination infection took place readily when the plants were exposed to a relative humidity of 96 percent or higher. At a relative humidity of 95 percent the percentage of infection was somewhat reduced, and at lower humidities no infection took place, irrespective of time. These results agree closely with those obtained by Fromme (*6*), Lauritzen (*21*), and others.

Data were likewise obtained on the drying of the foliage after atomizing with rust spores and on the effects of air movement on infection. It was found that infection readily took place when the moisture was allowed to evaporate from the leaves immediately after the application of spores to the plants, provided the plants were placed in an infection chamber with a saturated atmosphere. If, however, the dried plants were subjected to a relative humidity of 96 percent, no infection resulted or the amount was considerably reduced. An examination of plants held under these two conditions showed that in the one case moisture had condensed on the foliage and in the other it had not.

If plants are confined in a chamber at a relative humidity of 96 percent, with moisture already present on the foliage, it is possible for infection to take place before the droplets on the leaves have evaporated. Air movement also plays a part here. The above results were obtained without any artificial air movement. If the air is kept in circulation by means of a small fan blowing over the foliage, no infection takes place at 96 percent relative humidity. This can be

explained by the fact that the moisture on the leaves has evaporated before penetration takes place. On the other hand, in an atmosphere of 98 percent relative humidity or higher, air movement did not reduce the amount of infection.

RELATION OF LIGHT TO INFECTION

The foregoing experiments, designed for another purpose, did not lend themselves readily to a thorough investigation of the effect of light and high humidity on infection. A series of experiments was therefore designed in which young, vigorously growing plants were atomized with spores suspended in water and immediately placed in the infection chamber with a saturated atmosphere and subdued light. At daily intervals for a period of 6 days, several pots containing three plants each were removed from the infection chamber. Attempts were made to compare the intensity of the light within the chamber with that outside, but no satisfactory method was found, so this undertaking was finally abandoned. The infection chamber was placed in the greenhouse so that the temperature within it would approximate that on the outside where the plants were placed after their removal. In one series of experiments the door of the infection chamber was opened 24 hours after inoculation so that the humidity within would be about the same as that outside, where the plants were to be placed at the end of each removal period. In another experiment the door of the infection chamber was kept closed and the humidity high, and the plants were removed from it at intervals to another chamber differing only in being uncovered and thereby supplied with the normal amount of light.

Without detailing the results of these several series of experiments, it may be stated that they all pointed to the one general conclusion—that any abnormality in the functioning of the host is accompanied by delayed or reduced infection. Although normal infection was the rule after an exposure for 24 to 48 hours in an infection chamber, regardless of the light intensity, beyond that length of time not only was the percentage of infection reduced but the development of the pustules was noticeably retarded.

On plants removed from the dark infection chamber after 72 hours pustules developed about 24 hours later than on plants removed after 48 hours. In general this was true for each succeeding 24-hour period up to the end of 6 days, when the experiment was terminated. After about 48 hours in the infection chamber the plants began to show the results of this unfavorable environment by a slight yellowing of the foliage which became more pronounced as time went on. At the end of 6 days the plants were badly etiolated and some were wilted. Plants kept in the chambers for 5 or 6 days rarely recovered, and the percentage and rate of development of the rust pustules were correspondingly reduced. Plants confined for a shorter time in an environment of reduced light usually recovered, but the development of the sorus was correspondingly delayed. These data indicate, as other investigators have pointed out, that an environment unfavorable to the host is likewise unfavorable to the rust parasite.

TIME REQUIRED FOR INFECTION

Since humidity is vital to the germination of the spores and infection of the plant, its relation to the time required for germination and for infection to take place becomes increasingly important. In general, under field conditions there are comparatively few consecutive hours with a humidity of 95 percent or more, as figures 2, 3, and 4 show. (These charts, showing striking differences in climatic behavior, were selected as typical of the regions during the bean-growing season when rust infection would normally take place.) The fewer the hours required for infection the better is the chance for the rust to become established. In certain regions rains are frequent and dews are of almost daily occurrence, both of which contribute to the ease of establishing the disease. In the case of either dews or rains moisture would probably be deposited on the foliage for a sufficient length of time to permit germination of the rust spores.

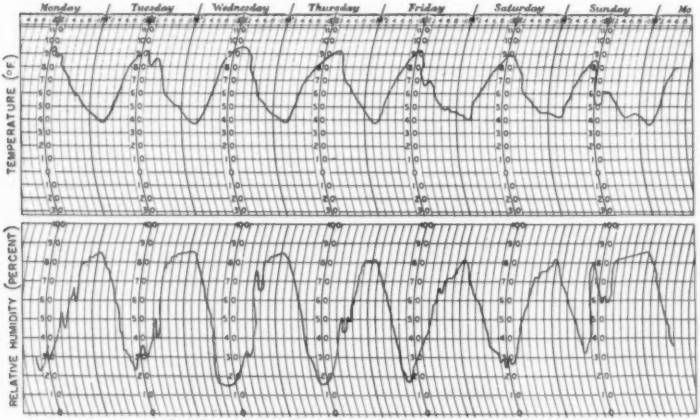

FIGURE 2.—Temperature and relative humidity at Greeley, Colo., over a period of 1 week, typical of the area during the bean-growing season when rust infections would normally take place.

On the other hand, in Colorado, for example, rains are infrequent and dews are rare, and the humidity is relatively low except for a few hours at night. It is in such regions that the outbreak of bean rust in an epidemic form cannot so easily be explained.

Controlled experiments conducted in an infection chamber under greenhouse conditions have been employed to determine the time required for infection to take place. The humidity of the infection chamber was held as near to saturation as possible, and in practically all cases there was a deposit of moisture on the foliage of the plants and on the walls of the infection chamber. The temperature varied somewhat but was held within the range favorable for infection. Vigorously growing young Pinto bean plants were atomized on both the upper and lower surface of the leaves with a water suspension of spores taken from young rust pustules. They were immediately placed in the infection chamber, which was covered in order to reduce the intensity of the sunlight. In duplicate experiments, two pots

with 2 to 4 plants each were removed at definite intervals from the infection chamber and placed in the greenhouse, where the temperature was approximately that of the infection chamber. The relative humidity of the greenhouse varied from 47 to 63 percent, so that the leaves dried in about 10 to 15 minutes after removal.

The results showed that no infection took place on plants removed from the infection chamber 2 to 6 hours after inoculation. Only very few pustules were formed on plants held in the infection chamber for 8 hours, but there was an appreciable increase at 10 hours. When the plants were in the chamber 12 to 18 hours infection was severe, and the results indicate that this length of time represented about the optimum. An important point in connection with the data is that under favorable temperature conditions some slight infection takes place within 8 hours.

It was noted also that after confinement of the plants in the infection chamber for 20 hours or more the percentage of infection was less

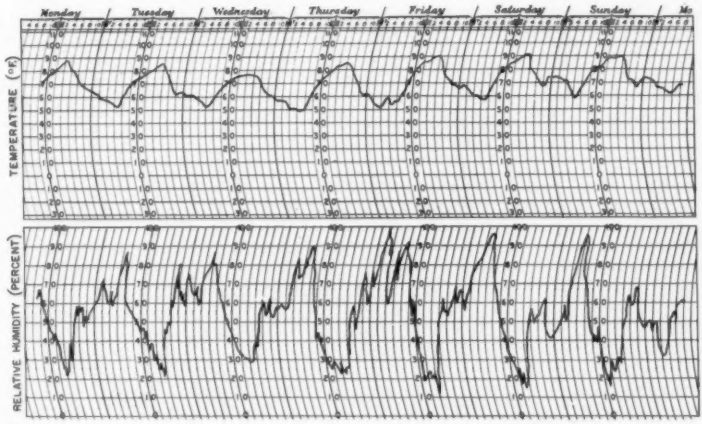

FIGURE 3.—Temperature and relative humidity at Twin Falls, Idaho, over a period of 1 week, typical of the area during the bean-growing season when rust infections would normally take place.

than after confinement for 12 to 18 hours. The decrease in the percentage of germination on plants confined in infection chambers for more than 18 hours is probably closely correlated with the condition of the plant.

Arthur (*2*), Ward (*30*), Raines (*25*), Stakman and Levine (*29*), Peltier (*24*), and others have emphasized the fact that there is a close relation between the vigor of the host and rust infection of cereals. In our experiments the plants were kept in subdued light for the entire length of the incubation period and in an almost saturated atmosphere, both conditions being abnormal. In consequence the reduced photosynthetic activities that prevailed were reflected in the vigor of the plant, and with this was associated a reduction in the percentage of infections.

CORRELATION OF WEATHER CONDITIONS WITH RUST OCCURRENCE AND EPIDEMICS

The bean rust epidemic in the Greeley, Colo., section in 1927 was the most severe that has been reported for the bean crop. Almost all the susceptible varieties were entirely destroyed, and those that were only partially resistant were badly damaged. On the other hand, no reports are known of rust ever occurring in southern Idaho. The situation in Colorado and Idaho is in sharp contrast to that in the coastal section of southern California, where rust epidemics occur every year. The question naturally arises, What are the conditions that result in a rust epidemic occasionally in Colorado, never in Idaho, and yearly in southern California? Probably because of the many factors involved, the question cannot be answered with certainty, but both temperature and humidity doubtless play important parts. Any data collected are seemingly too incomplete to account

FIGURE 4.—Temperature and relative humidity at Chula Vista, Calif., over a period of 1 week, typical of the area during the bean-growing season when rust infections would normally take place.

for the exceptions and variations from season to season and between regions widely separated geographically. The source of the inoculum, the condition of the host, and favorable temperature and humidity for germination of the spores are doubtless important factors that contribute to the initial infection. Conditions favorable to the production of a second and probably a third generation of spores would perhaps be required to multiply them in sufficient numbers to create an epidemic. Experiments have shown that 8 to 10 days are required from the time of inoculation for urediospores to mature. On this basis about 3 weeks would be required to spread the infection sufficiently to constitute an epidemic. If, however, the proper environmental conditions, such as temperature and humidity, did not coordinate with spore dissemination, succeeding generations would fail and no epidemic would materialize. The conclusion is probably warranted that during those years when no rust occurs or when it occurs only to a limited extent the chain of favorable conditions for

multiplication and dissemination of the spores is broken sometime during the summer, before the infection reaches the epidemic stage.

With the hope of obtaining some information on the epidemiology of bean rust, constant temperature and humidity records were taken during the summer months of 1929 and 1930 at Greeley, Colo. (fig. 2), Twin Falls, Idaho (fig. 3), and Chula Vista, Calif. (fig. 4). As inaccurate as such records conceivably may be, certain facts are revealed that may contribute to a partial explanation of why the three areas differ so markedly with respect to the occurrence of bean rust.

Controlled experiments have shown that the urediospores of the bean rust infect best at a temperature of about 17° C. It was also demonstrated by similar experiments that no infection takes place at a humidity below 95 percent, the optimum being near saturation. To insure infection it was necessary to hold the plants under these conditions for at least 8 hours, and from 12 to 18 hours or longer were required for optimum results. With these facts in mind, it is interesting to compare the three hydrothermographic chart records (figs. 2, 3, and 4) with respect to daily temperatures and relative humidities. These charts record the results for only a single week, but they were selected from a large number as typical of the three areas and indicate differences that are more or less constant.

It will be seen that in southern California (fig. 4) the proper temperature and humidity requirements for spore germination and for infection occur daily. At night the humidity approximates saturation for 8 to 12 hours, and the temperature is at the optimum for rust infection. A fog regularly sweeps in from the sea during the night and often remains until 9 or 10 a. m., and during this time the foliage is wet. Under such ideal conditions bean rust infection occurs regularly every year, even though rains rarely occur during the summer months. Quite different conditions prevail in southern Idaho (fig. 3), except that, as in southern California, summer rains are rare. The temperature is high in the daytime, but at night it is within the optimum for infection. Figure 3 shows that, while the relative humidity may reach 95 percent or more for a very short time during the night, it does not last long enough to allow the spores to germinate and infection to take place. An analysis of figure 3 with respect to temperature and relative humidity would suggest that bean rust would probably never become established in southern Idaho.

The record for Greeley, Colo., falls between those of Chula Vista, Calif., and Twin Falls, Idaho. Rain may occur at any time during the growing season, although long rainless periods occur every summer. The usual night temperatures are optimum for infection, but the relative humidity is extremely variable at night and usually is not high enough to permit infection, although it may occasionally occur. Such high humidities might be expected now and then to correspond in the matter of time with the dissemination of the rust spores. The occasional high humidities, together with rainy periods lasting several days and coming at just the proper time, might account for the occasional epidemics of bean rust in that area.

The absence of a primary source of spore material can hardly be regarded as a limiting factor. Neither can the abundance of spore material insure a rust epidemic the following year. It is the practice in Colorado to stack the bean straw after threshing, feed it to sheep,

or scatter it on the fields. In 1928, after the epidemic of 1927, abundant spore material was found in the straw stacks throughout the area. Teliospores were abundant in the straw and were viable into the summer of 1928. Yet in 1928 there was only a slight amount of rust, distributed more or less locally, which suggests that the proper environmental conditions did not occur or that they were not correlated with the generations of rust development.

VARIETAL SUSCEPTIBILITY AND RESISTANCE

Data on the susceptibility of beans to rust are contained in publications by Fromme and Wingard (*8*, *9*), Morse,[4] Gassner (*11*), and Jordi (*16*). Fromme and Wingard inoculated in the field and greenhouse a large collection of varieties and determined their relative susceptibility by using the Tennessee Green Pod, one of the very susceptible varieties, as a standard for comparison. Morse lists a number of varieties with respect to their susceptibility to rust in Maine. Gassner carried out inoculations in South America and found considerable difference in the susceptibility of varieties. Jordi took field notes on 5 pole varieties in Europe and found 4 to be very susceptible and 1 quite resistant.

Fromme and Wingard adopted the scheme of grading the Tennessee Green Pod 100 percent susceptible and comparing other varieties with it. Those showing greater susceptibility than the Tennessee Green Pod were graded proportionately above 100, and those less susceptible were graded correspondingly less.

In the course of investigations of bean rust and other bean diseases the writers had occasion to make a large collection of domestic and foreign varieties with the hope that some of them might be of suitable type and show sufficient resistance to rust and other diseases to be used as parents in the production of resistant strains. Many lots of seed were received from China, Union of Soviet Socialist Republics, Germany, Mexico, Chile, Venezuela, Rhodesia, Uganda, Puerto Rico, Iran, Japan, and Guatemala, through the Division of Plant Exploration and Introduction, Bureau of Plant Industry, United States Department of Agriculture. Most of these varieties were unidentified as to name but were designated by a serial number. Many lots were received independently from foreign countries and were often mixed and unnamed. All these were tested for rust resistance. The experiments on susceptibility of most of the domestic varieties were carried out in two localities, namely, in the greenhouse at Washington, D. C., and in the field at Chula Vista, Calif., where rust epidemics occur annually. While artificial inoculation was practiced once or twice in the field tests, it was not generally necessary. Since the season in California is sufficiently long to grow two crops, one test was made in the spring and one in the fall. Greater infection occurred in the fall than in the spring. The data from the field and greenhouse experiments are shown in table 1.

Many of the varieties tested by Fromme and Wingard were included in the present collection. The results on the whole agreed very closely, although their tests were made under somewhat different climatic conditions.

[4] UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF PLANT INDUSTRY. RUST CAUSED BY UROMYCES APPENDICULATUS. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Bull. 2:174. 1918. [Mimeographed.]

Another system of grading resistance and susceptibility has been employed, that of Jordi, which consists in arranging the varieties in a scale from 0 to 10, immune varieties being graded 0, the most susceptible 10, and intermediate forms between these two extremes. The Kentucky Wonder was used in California as a standard of comparison because of its extreme susceptibility to rust. On the other hand, the Pinto was used mostly in the greenhouse tests at Washington, D. C.

The susceptibility or resistance of the varieties from foreign sources was determined by greenhouse inoculation. Where it was possible to segregate the mixtures according to color, shape, and other characters of the seed, infection experiments were conducted. In cases where the samples could not be identified with certainty, the results are not recorded in the table. Suffice it to say that practically all the unidentified foreign lots showed some degree of susceptibility.

TABLE 1.—*Bean varieties arranged according to degree of rust susceptibility under field conditions in southern California, together with infection ratings in greenhouse tests at Washington, D. C.*

Group, susceptibility, and variety	Infection rating [1]	
	Field results [2]	Greenhouse results [3]
Field beans:		
Very susceptible:		
California Pink	10	9
California Red	10	9
Pinto	10	10
Red Mexican	10	9
Blue Pod Small White	9–10	9
Large White	9–10	9
Great Northern	7–10	9
Michigan Pea	7–9	8
Moderately susceptible:		
Genuine Small White	6–8	9
Tepary (*Phaseolus acutifolius latifolius* Freeman)	6–7	5–10
Robust	5	9
Slightly susceptible:		
Bayo	2–4	5
White Kidney	3	3–8
Brown Swedish	2–3	-------
Red Kidney	2–3	7
Yellow Eye	2	-------
Large White Marrow	1–2	7
Cranberry	0	0
Garden beans:		
Very susceptible:		
Kentucky Wonder (brown-seeded)	10	10
McCaslan	8–10	-------
Tennessee Green Pod	7–10	10
Moderately susceptible:		
Striped Creasback (Scotia)	6–8	9
Golden Cluster Wax	6–7	4–7
Kentucky Wonder (white-seeded)	5–7	3–9
Kentucky Wonder Wax	6	2–0
Dutch Case Knife	4–6	9
Garden Beans—Continued.		
Slightly susceptible:		
Burpee Brittle Wax	3	2
Burpee White Wax	3	-------
Fordhook Favorite	2	2
French Horticultural	2	2
Improved Kidney Wax	2	3
Low Champion	2	2
Rust Proof Golden Wax	2	-------
Late Stringless Refugee	2	2
Keeney Rustless Wax	1–2	1
Hodson Wax	0–4	2
Longfellow	0–4	5
Red Valentine	0–4	7
Bountiful	0–3	4
Black Valentine	0–3	3
Burpee Stringless Green Pod	0–3	4
Currie Rust Proof Wax	0–3	2
Full Measure	0–3	5
Pencil Pod Black Wax	0–3	3
Refugee Wax	0–3	-------
Wardwell Kidney Wax	0–3	3
Black Wax	0–2	3
Davis White Wax	0–2	7
Giant Stringless Green Pod	0–2	3
King Mammoth Horticultural	0–2	2
Refugee 1000–1	0–2	2
Sure Crop Wax	0–2	3
Canadian Wonder	0–1	7
Low Champion Bush	0–1	-------
Improved Golden Wax	0–1	3
Long Yellow Six Weeks	0–1	2
Mohawk	0–1	2
Refugee (extra early)	0–1	2
Round Pod Kidney Wax	0–1	2
Lazy Wife	0	1
Weber Wax	-------	1

[1] On scale of 1 to 10.
[2] Brown-seeded Kentucky Wonder, graded 10, used as standard of comparison. Notes by I. C. Jagger.
[3] Pinto, graded 10, used as a standard of comparison. Notes by C. F. Andrus.

In general there was appreciable injury to the varieties when the rust reaction was scored 5 to 10, but little or none when scored below 5. Probably varieties scored below 5 would be expected to suffer from rust only when exposed to very severe and heavy infection. A score of 1 indicates that a trace of rust was perceptible only on close examination. It has been found that the most resistant varieties may be expected to show a trace of rust under very favorable conditions.

A careful examination of table 1 shows that, out of a total of 61 varieties listed, only 19 show under field results a degree of susceptibility that would be regarded as positively destructive; 7 of these are pole varieties, 11 field beans, and 1 (Tennessee Green Pod) a bush bean.

The percentage of highly susceptible varieties among the so-called field and pole beans (field results) is relatively high. Among the pole beans only 2 varieties (Lazy Wife and King Mammoth Horticultural) showed any marked degree of resistance, while 7 out of 18 field bean varieties would be considered sufficiently resistant to recommend for growing in localities where rust epidemics occur.

Twelve varieties of lima bean (*Phaseolus lunatus macrocarpus* Benth.) grown in the United States and obtained from commercial sources, 1 variety from China, and 2 from Japan were tested for rust resistance by inoculation in the greenhouse, with the following results:

Variety [5]	*Rating*
Carpenteria	8
Challenger	8
Dreer	8
Early Leviathan	8
Emerald Isle	8
Evergreen	8
Fordhook	8
Henderson	2
King of the Garden	8
Sieberts	8
Sieva	2
Wood Prolific	2
28805 (from Japan) [6]	3
28807 (from Japan) [6]	3
29067 (from China) [6]	2

All the domestic varieties except 3 (Wood Prolific, Sieva, and Henderson) are graded 8 in susceptibility and therefore fall in the class of very susceptible varieties. Considerable damage to most of the varieties might be expected when they are grown in localities where rust is likely to develop. The three lots or varieties from foreign sources showed considerable resistance to rust, as their ratings of 2 and 3 indicate.

PHYSIOLOGIC FORMS

The investigations on bean rust indicated a considerable difference in the relative susceptibility and resistance of different varieties. Some were extremely susceptible, and urediospores were abundantly formed in the sori. In the case of susceptible varieties a ring of secondary sori, and frequently even a third ring, develops outside the

[5] The seed of the 12 domestic varieties was obtained from commercial sources. The writers have used the trade names without regard to possible synonomy.

[6] Seed obtained from D. N. Shoemaker, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry.

primary sorus, so that a spot of considerable diameter finally results on both the upper and lower surfaces of the leaf. On the other hand, in partially or almost completely resistant varieties, abortive sori, their size and extent depending on the degree of resistance of the host, are produced, in which few or no spores develop. Such abortive sori are often almost invisible to the unaided eye. The fungus penetrates the host tissue generally, growing through the leaf and forming a lesion on both the upper and the lower surface. The epidermal cells are killed and collapse, so that a slightly sunken area is formed on the surface. In some cases the infection spots suggest a corrosion of the epidermis with later collapse of the epidermal cells. A few spores may be formed on the lower leaf surface but rarely in the dorsal lesions.

In testing the resistance and susceptibility of beans to rust several German varieties of the Kentucky Wonder type were inoculated with a rust from southern California and with one collected in the vicinity of Washington, D. C. Only sterile abortive flecks developed on some of these varieties, showing that they possessed considerable resistance. However, a few scattered, normal, sporulating sori were found on practically all the plants of the resistant varieties. These sori were typical of those found on plants of susceptible varieties, from which it was concluded that two physiological forms of the bean rust composed the original culture, one being present in an extremely small proportion. For the sake of clarity the parent culture was designated form 1 and the rare culture form 2. Form 2 was isolated by the inoculation of additional plants of the same varieties with spores from the normal sori, and the results were compared with the results of the parallel inoculations with form 1. Form 2 gave good infection, with the production of normal spore-producing sori, while form 1 gave the usual flecking and a few normal sori, which persisted probably as a contamination of the parent culture. These experiments were repeated several times with the result that the difference in virulence of the two forms was maintained throughout.

Form 2 was isolated from bean rust material from two widely separated places—California and Washington, D. C. The two original cultures are pathogenetically alike when inoculated on the same varieties of beans, but there are reasons to believe that they differ genetically. The rust of California origin does not include the telial stage and therefore must have been without sexual reproduction for at least many generations. On the other hand the rust culture collected in the East normally produces telia both in the field and under greenhouse conditions. It appears, therefore, that both forms 1 and 2 are represented by teliospore-producing and non-teliospore-producing strains. In other respects the two forms appear to be morphologically alike.

The data recorded in table 2 call for some explanation. Kentucky Wonder Wax, white-seeded Kentucky Wonder, and brown-seeded Kentucky Wonder are domestic varieties and are cultivated extensively in different sections of the United States. The green-podded Kentucky Wonder varieties grown in the United States are quite susceptible to rust; although the one with white seed was claimed to be resistant in California, it was found not to be when subjected experimentally to rust infection. On the other hand the Kentucky Wonder Wax was extremely variable, some plants being almost immune to form 1, while others were susceptible. All plants were very sus-

ceptible to form 2. The data from this variety would indicate that it is not a pure line, or at least not entirely fixed so far as its relation to rust infection is concerned.

TABLE 2.—*Host reactions of certain pole beans of the Kentucky Wonder type to physiologic forms 1 and 2 of bean rust*

Variety	Form 1		Form 2	
	Characterization	Rating [1]	Characterization	Rating [1]
Basketful	Susceptible	10	Susceptible	10
Erntebringer (Phaenomen)	do	10	do	10
Hildesheimer (Phaenomen)	Resistant	2	do	10
Kentucky Wonder (brown-seeded)	Susceptible	10	do	10
Kentucky Wonder Wax	Variable	2–10	do	10
Kentucky Wonder (white-seeded)	Susceptible	10	do	10
Meisterstuck	Resistant	2	do	10
Meisterstuck (selected)	Susceptible	10	do	10
Phaenomen	Resistant	2	do	10
President Roosevelt	Susceptible	10	do	10
Schlachtschwert (original)	Variable	2–10	do	10
Schlachtschwert (selected)	Resistant	2	do	10
Do	Variable	2–10	do	10
Do	Susceptible	10	do	10
World Wonder	Resistant	2	do	10
Do	Susceptible	10	do	10
Zeppelin Giant	Resistant	2	do	9
Zeppelin Mulstopper	do	2	do	10

[1] Varieties graded 0 or 1 are practically immune; those graded 10 are very susceptible.

An examination of table 2 shows several of these varieties to be almost entirely immune to bean rust form 1 and very susceptible to form 2. The results with World Wonder and Schlachtschwert call for special comment. The World Wonder was received in two lots; one lot shows decided resistance and the other a high degree of susceptibility to form 1, while both are very susceptible to form 2. Schlachtschwert is even more interesting, since the original lot contained seed that produced plants varying from 2 to 10 in susceptibility to form 1; no resistance was apparent to form 2. This variety has been cultivated for a few years and selections have been made from it on the basis of marketable type. Out of these selections there were obtained 1 strain resistant and 1 susceptible to form 2 and 1 that varied from 2 to 10 in susceptibility. This variety apparently is not genetically fixed when its tolerance to rust infection is considered.

Form 2 appears to be the more virulent strain and readily infects varieties that are partially to almost completely resistant to form 1. Form 1 apparently is the more widely distributed strain and greatly predominates in mixtures of the two.

After most of the studies with the two rust forms had been completed, an occasional plant of the Kentucky Wonder Wax was found to resist infection by both forms 1 and 2. The seed from the resistant plants were saved and multiplied. Additional inoculations with both strains of rust demonstrated the existence of an inherent resistance and not, as might be at first suspected, a failure to obtain infection.

No attempt has been made to exhaust the possibilities of physiologic forms. The writers feel that additional forms could probably be isolated if collections of rust were made from widely separated regions of the world and tested on a number of differential hosts. A large

number of forms have been reported for cereal rusts (*3*), and it is not unlikely that additional forms occur on the bean.

RUST CONTROL

In respect to the problem of prevention and control, bean rust is not unlike rusts of other crops. It is prevalent when conditions are ideal for its development, and it is difficult to control except by the planting of tolerant or resistant varieties or by the development of resistant strains by breeding and selection.

Investigations on the control of rust by the use of fungicides have been numerous. Kellerman (*17*), Pammel (*22*), Galloway (*10*), and others carried out spraying experiments for the purpose of controlling stem rust of oats and leaf rust of wheat, oats, and barley, but with little success. More recently several investigators, among whom may be mentioned Kightlinger (*18*), Bailey and Greaney (*5*), Lambert and Stakman (*20*), and Greaney (*13*), have attempted cereal rust control by the use of sulphur dusts. The results on the whole have been fairly consistent in demonstrating the effectiveness of sulphur in preventing rust infection. The decrease in the percentage of rust was accompanied by a corresponding increase in yield. Different methods of application were employed, and the general conclusion seems to be that the rust could be controlled if a practical method of applying the dust could be devised. The time of application with respect to infection and rains was found to be important.

GREENHOUSE EXPERIMENTS

The possible value of dusting with sulphur compounds to control bean rust, as shown by preliminary field trials, suggested the desirability of carrying out experiments in the greenhouse, where conditions such as temperature and humidity could be controlled adequately. It was also believed that the results of such trials might be useful when an opportunity was afforded to conduct additional field experiments. After inoculation the plants were confined in an infection chamber, the temperature of which was held within a range previously shown to be most favorable for infection. The humidity was held near to saturation. Commercial products known as Kolodust and flowers of sulphur were used, and no difference in results between the two could be observed. Young, healthy plants of the Pinto variety were used for all the dusting experiments. Some plants were dusted immediately before and some immediately after the leaves had been atomized with rust spores, and some after different intervals of time following inoculation. Perfect control was obtained whether the plants were dusted immediately before or immediately after inoculation.

These results were followed by experiments to determine how long after the plants are inoculated the dust may be applied and still be effective. Considerable interest was attached to these experiments, not alone from the standpoint of control but because it was hoped that they might give some information as to the time that elapses after the application of the rust spores before penetration takes place. A preliminary series of experiments was initiated in which a number of plants were atomized with spores and confined in an infection chamber. For a period of 5 days after inoculation

several plants were removed from the chamber every 24 hours. These plants were dusted with flowers of sulphur. The application of the sulphur had no influence whatever, the rust pustules being just as numerous on the dusted plants as on the controls. These results indicate that penetration of the host tissue had taken place in the first 24 hours. The results showed also that the plants confined in the infection chamber the longest were proportionately later in developing pustules, a condition for which the dust is not responsible but which can be attributed to the environment that was presumably unfavorable for the development of the plant and the rust.

Dusting the plants at intervals of 6, 12, and 17 hours after inoculation was next tried. Controls dusted immediately after inoculation and some not dusted were run in all the experiments. Good rust infection was obtained with the undusted controls. On the other hand, perfect control was obtained when the plants were dusted immediately after atomizing. Dusting 6 hours after inoculation gave perfect control. A few rust pustules developed on plants dusted after 12 hours, and good infection developed after a 17-hour period, though it was not quite equal to that of the undusted controls. These results show that no penetration, at least none beyond the possibility of injury by the sulphur, had taken place at the end of 6 hours. On the other hand some of the germ tubes had safely penetrated the host in 12 hours, and many of them at the end of 17 hours.

The results obtained with sulphur as a control of bean rust suggested trials with a copper-lime dust. A commercial preparation consisting of 20 parts copper sulphate and 80 parts hydrated lime was dusted on one group of plants before inoculation, and on another at intervals of 3, 6, 17, 20, 24, and 41 hours after they had been sprayed with spores (form 2). The dusted as well as the inoculated undusted controls were confined in an infection chamber for 42 hours and then removed. The results of these experiments suggested that very inadequate control, if any, could be expected from dusting with a copper-lime dust. No benefit resulted from dusting immediately before inoculation, and only a very limited reduction in infection was obtained when the plants were dusted immediately after inoculation. Similar results were obtained from repeated experiments. Judging from these results, dusting with sulphur offers more promise of control than copper-lime dust.

FIELD EXPERIMENTS

In 1928, after the 1927 epidemic of bean rust in Colorado, some field experiments were initiated in its control. There was only a limited amount of rust that year, but enough to give some idea of what might be expected should control measures be applied at the right time. The dusts used in 1928 were commercial preparations, consisting of different forms of oxidizing sulphur and one known commercially as Kolodust. There were three control plots. On July 31 there was a little rust in evidence when an application was made with a hand duster at the rate of 5 pounds per one-fourteenth of an acre. A second application was made August 7. The latter part of the season was not particularly favorable for rust development, which increased only moderately after the second dusting, so a third application was not found necessary. On September 3, final records were taken of the experiments, and it was found that 15 to

25 percent more damage had been done to the controls than to the dusted plots. Had it been a bad rust year, better results might have been obtained. The control plots yielded a fair crop. Since 1928 so little rust has occurred that no further experiments have been carried out in the field.

No general conclusions can be drawn from these experiments, but they suggest that control of rust may be possible if a practical way can be found to apply the dust. The control of bean rust by the application of liquid or dust fungicides, while possible from a seasonal standpoint, is at most only temporary and of doubtful practicability.

A number of varieties that resist the rust sufficiently for practical purposes have been tested. There is more resistance exhibited by the snap bean varieties than by the field beans. Unfortunately, the more popular and widely grown field varieties are very susceptible. Breeding and selection must be employed in order to secure substitutes for susceptible varieties that will be capable of producing a crop in rust-infected areas. In fact the evidence indicates that the control problem will never be adequately solved until resistant varieties are developed by hybridization and selection.

SUMMARY

Observations on the succession of spore forms indicate that the bean rust (*Uromyces phaseoli typica* Arthur) can be made to complete its full life cycle under greenhouse conditions.

Recognition is made of a teliospore-producing and a non-teliospore-producing strain of bean rust. Attempts to force teliospore formation reveal that the abundance of teliospores, within the teliospore-producing strain, as well as the time when they appear, may be controlled to an appreciable extent by altering host metabolism.

Teliospores 207 days old and urediospores 182 days old, were germinated. Urediospores show a decrease in germinability with age, while teliospores gave maximum germination after 6 months.

A brief comparison is made of the bean rust with the cowpea rust (*Uromyces phaseoli vignae* (Barcl.) Arthur) and rust of *Strophostyles helvola*.

The optimum temperature for spore germination in bean rust was found to be about 14.5° C., determined at the end of a 24-hour period, on a percentage basis. The optimum temperature for infection was slightly higher than that for spore germination, or approximately 17°.

No infection took place at a relative humidity below 95 percent. Infection seems to require either moisture condensation or an initial excess of moisture on the plants at the beginning of the infection period.

The presence or absence of light influenced infection. Plants sprayed with spores and confined in a moist chamber in subdued light or almost total darkness for 24 to 48 hours became normally infected. If confined for a longer time, the number of sori was somewhat reduced and their development delayed. The results point to the conclusion that an abnormality in the functioning of the host is accompanied by delayed or reduced infection.

The minimum time for infection to take place under optimum conditions is 8 hours; 12 to 18 hours constitutes about the optimum time.

Results indicate that outbreaks of bean rust are possible in any region where a relative humidity of 95 percent or more is maintained for any period of 8 hours or more.

Sixty varieties of garden and field beans (*Phaseolus vulgaris*), 15 varieties of the lima bean (*P. lunatus macrocarpus*), and the tepary bean (*P. acutifolius latifolius*) were tested for rust susceptibility and rated on a scale of 0 to 10.

A new physiologic form of bean rust was discovered and designated form 2.

Dusting with sulphur suggests an effective means of controlling bean rust.

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THE MICROBIOLOGICAL DECOMPOSITION OF THE CONSTITUENTS OF ALFALFA HAY [1]

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INTRODUCTION

Investigation of the biological, chemical, and engineering aspects of the spontaneous ignition of hay and similar agricultural products has been under way in the Bureau of Chemistry and Soils for several years [3] (*2, 5, 6, 12, 13, 14, 15, 27, 31*).[4]

It is now recognized that micro-organisms play an important role in the early stages of the decomposition which finally leads to the spontaneous ignition of plant material (*7, 18*). However, little detailed information can be found regarding the disappearance of the constituents of alfalfa hay when subjected to microbial decomposition either under laboratory conditions or in a heating haystack. The results of a laboratory study of this problem are presented in the present paper.

REVIEW OF LITERATURE

During recent years the literature on the microbiological decomposition of lignified plant materials has rapidly increased. Soil chemists and soil bacteriologists have been particularly active in studying this problem in the hope of gaining a better understanding of the mechanism involved in the formation of soil organic matter or humus. No attempt will be made here to review this literature as this has already been done elsewhere (*8, 23, 25*). It is necessary only to point out that there appears to be a decided difference, insofar as the action of fungi and bacteria is concerned, between lignin as it occurs naturally in plants and free or isolated lignin. Willstätter lignin, or lignin isolated by the 72-percent-sulphuric-acid method, appears to be resistant to the action of soil micro-organisms and to the wood-destroying fungi *Trametes pini* and *Polystictus hirsutus* (*11, 30, 35*). Pringsheim and Fuchs (*26*), and more recently Demme (*8*), have shown that lignin isolated by the alkali method can be demethoxylated by soil micro-organisms. Under aerobic conditions natural lignin can be decomposed by soil micro-organisms, at least in part, although the rate of decomposition is generally less than that of cellulose and hemicelluloses (*3, 25, 34*). Recently Boruff and Buswell (*4*) reported that they were unable to ferment isolated lignin under anaerobic conditions, though the natural lignin in cornstalks could be fermented under these conditions. This is in opposition to the previous findings of Tenney and Waksman (*32, 33*), who

[1] Received for publication Jan. 28, 1935; issued July 1935. This paper is a joint contribution from the Color and Farm Waste, Chemical Engineering, and Food Research Divisions of the Bureau of Chemistry and Soils.

[2] To C. A. Browne, chief of Chemical and Technological Research of this Bureau, who initiated this investigation, the authors wish to express their thanks for helpful suggestions made in the course of the work.

[3] U. S. DEPARTMENT OF AGRICULTURE, BUREAU OF CHEMISTRY AND SOILS. REPORT OF CONFERENCE ON SPONTANEOUS HEATING AND IGNITION OF AGRICULTURAL AND INDUSTRIAL PRODUCTS, CALLED BY NATIONAL FIRE PROTECTIVE ASSOCIATION, IN COOPERATION WITH U. S. DEPARTMENT OF AGRICULTURE AND U. S. DEPARTMENT OF COMMERCE, WASHINGTON, D. C., NOV. 14 AND 15, 1929. 127 pp. [Multigraphed.]

[4] Reference is made by number (italic) to Literature Cited, p. 773.

reported that under anaerobic conditions the natural lignin of alfalfa was preserved practically quantitatively. In a recent article Juon (*16*) reported that green fodders subjected to fermentation suffered a loss of lignin. In most cases, however, the loss was rather small. In general, it was found that lignin was more resistant to the action of micro-organisms than the other major plant constituents.

EXPERIMENTAL PROCEDURE

In studying the microbiological decomposition of alfalfa hay in the laboratory, two types were selected, one representing the first crop of the season (referred to in this paper as "first cutting"), and the other representing the third crop ("third cutting"). The samples of each cutting were collected fresh from the field, sun-dried for several days, then ground fine in a mill. For analysis a subsample was dried in an oven at 105° C. The following determinations were made. The percentage of ash was calculated on the basis of oven-dried material, and all the other results were calculated on the basis of oven-dried, ash-free material.

Ash.—This was determined in the usual manner by igniting a weighed sample with a bunsen burner and weighing the inorganic residue.

Alcohol-benzene extractives.—These were determined by extracting a weighed sample in a Soxhlet extractor for 30 hours with a 1:2 alcohol-benzene solution and ascertaining the loss in weight.

Cold-water extractives.—To a weighed sample of the dry material which had been extracted with the alcohol-benzene solution, distilled water was added in the proportion of 150 cc to 1 g of sample and allowed to digest at room temperature, with frequent stirring, for 48 hours. The loss in weight after extracting and redrying was calculated on the basis of the original unextracted ash-free material.[5]

Hot-water extractives.—A weighed sample of the dry residue from the previous extraction was treated with distilled water (in the proportion of 150 cc of water to 1 g of sample) and boiled under the reflux condenser for 3 hours. The loss in weight was calculated on the basis of the original dry unextracted ash-free material.[5]

Uronic acid anhydrides.—These were determined in the dry unextracted material according to the procedure recommended by Dickson, Otterson, and Link (*9*) as modified slightly by Phillips, Goss, and Browne (*24*). The percentage of uronic acid anhydrides in a plant material is a measure of its total polyuronide content. They may be present in pectins or in hemicelluloses.

Pentosans.—The percentage of furfural in the dry unextracted sample was determined by the method of the Association of Official Agricultural Chemists (*1*). One-sixth of the percentage of uronic acid anhydrides was deducted from this, and the result was calculated as percentage pentosans.

Cross and Bevan cellulose.—This was determined by the method of Norman and Jenkins (*20*), but instead of the filter suggested by these authors, Jena sintered glass crucibles were employed.

Furfural in Cross and Bevan cellulose.—This was determined by the method of the Association of Official Agricultural Chemists (*1*). The phloroglucide precipitate was washed with 100 cc of ethanol

[5] In view of the fact that the water-soluble ash was rather small, no attempt was made to determine the percentage of ash in the extracts.

(heated to 60° C.) to remove any phloroglucide of methyl-furfural present.

Cellulose.—The Cross and Bevan cellulose consists essentially of two components, a true cellulose fraction and a furfural-yielding fraction, which in all probability is xylan (*19*). In order to ascertain the true cellulose content of the material, the percentage of furfural in the Cross and Bevan cellulose was determined, as already indicated, and the result calculated as percentage pentosans. The result thus obtained when deducted from the percentage of Cross and Bevan cellulose gave the percentage cellulose in the sample. Thus, if C equals the percentage of Cross and Bevan cellulose, P the percentage of pentosans in the Cross and Bevan cellulose, the percentage of cellulose equals $C-(C\times P/100)$.

Lignin.—The weighed sample which had been successively extracted with a 1: 2 alcohol-benzene solution, cold water, and hot water, according to the procedures described above, was treated with a 1-percent hydrochloric acid solution, in the proportion of 1 g of plant material to 150 cc of acid, and boiled under the reflux condenser for 3 hours. The insoluble residue was filtered off, and dried at 105° C., and the loss in weight was determined. The extracted material was analyzed for lignin by the fuming hydrochloric acid method according to the procedure described by Phillips (*22*). Three samples were weighed out, and in the lignin residues from the first two samples the percentages of ash and nitrogen were determined, and in the lignin fraction from the third sample the percentage of methoxyl was determined. Corrections were made for the nitrogen and ash in the lignin.

Methoxyl in ash-free lignin.—The lignin residue obtained from the third sample used in the determination of lignin was collected in a Pyrex sintered glass crucible (2 cm inside diameter and 5 cm high). The weight of lignin, corrected for ash and crude protein, was determined. The percentage of methoxyl in this was determined by the procedure described by Phillips (*22*). The methyl iodide was absorbed in pyridine as recommended by Kirpal and Bühn (*17*). A slightly modified Zeisel apparatus (*22*) was used. The reaction flask consisted of the outer cone of a no. 30 interchangeable ground glass joint, the unground end of which had been sealed off and rounded out. By means of this arrangement it was possible to insert the glass crucible containing the lignin into the reaction flask. To the condenser of the Ziesel apparatus, the inner cone of a no. 30 interchangeable ground glass joint was sealed.

Methoxyl in unextracted plant material.—The percentage of methoxyl in the dried unextracted plant material was determined as described above. The methyl iodide was absorbed in pyridine.

Methoxyl in extracted plant material.—The method generally used for the determination of the percentage of methoxyl in an organic substance is based on a reaction which all ethers undergo when heated with hydriodic acid. The reaction may be represented as follows: $ROR'+HI=ROH+R'I$, in which R and R' may be either alkyl or aryl groups. If R' is a methyl group, that is, if the substance contains a methoxyl group, then methyl iodide is formed as a result of the reaction, and this may be absorbed either in pyridine or in an alcoholic silver nitrate solution and quantitatively estimated. However, if methyl esters are also present, these two react with hydriodic

acid and yield methyl iodide. Accordingly, when a plant material is heated with hydriodic acid not only do the methoxyl groups of methyl ethers react but also those in esterlike combination, as in the case of pectins and in methyl esters of organic acids generally. If the plant material is subjected to a preliminary hydrolysis with dilute acid, however, the methoxyl groups attached in etherlike combination are very little affected by this treatment, whereas the methyl groups of methyl esters are split off. It is now known definitely that the methoxyl groups in lignin are attached in etherlike combination, so that by determining the percentage of methoxyl in a lignified plant material, such as alfalfa which has been subjected to a preliminary hydrolysis with dilute acid, the percentage of methoxyl obtained represents essentially, although perhaps not entirely, lignin methoxyls. It will be observed that the weight of methoxyl in the extracted plant material is in every case greater than the actual quantity of methoxyl in the lignin (weight of ash-free lignin×percentage of methoxyl in the lignin). This difference is small, however, and may be due either to (1) a partial demethoxylation of the lignin brought about in the course of its isolation from the plant material, or (2) to firmly bound methoxyl groups occurring in some component of alfalfa other than lignin. It has been shown by O'Dwyer (*21*), Schmidt and his coworkers (*29*), and by Hägglund and Sandelin (*10*) that in wood some firmly bound methoxyl groups are also in the carbohydrate fraction. The determination of the percentage methoxyl in a lignified plant material that had been treated in the manner described above, however, does give an approximate idea of the quantity of lignin present. This determination was carried out as follows: The weighed sample was successively extracted with a 1: 2 alcohol-benzene solution, cold water, hot water, and a 1-percent hydrochloric acid solution under conditions previously described, and the loss in weight was determined. The percentage of methoxyl in the extracted sample was determined by the method already described in the paragraph headed "Methoxyl in plant material", and the result calculated on the basis of the original unextracted ash-free material.

Crude protein ($N \times 6.25$).—This was determined in the unextracted plant material in the usual manner by the Kjeldahl, Gunning, Arnold method (*1*).

The terms "pentosans", "lignin", and "cellulose" as well as those used for the other constituents in this paper refer only to plant substances or plant fractions as defined by the analytical methods employed.

In all the experiments both first-cutting and third-cutting alfalfa were used. In every experiment sun-dried ground hay was employed (equivalent to 20 g oven-dried (105° C.) material). The several samples were placed in Erlenmeyer flasks, and to each a sufficient quantity of a modified Czapek's solution was added as the nutrient medium. In place of the sodium nitrate in the regular Czapek solution an equivalent quantity of urea was substituted, and the sugar generally used was omitted. In a previous investigation by Phillips, Weihe, and Smith (*25*) it was found that when this modified Czapek's solution was used the reaction of the medium remained almost neutral throughout the experiment. In all experiments conducted under aerobic conditions the pH of the medium after fermentation ranged from 7.0 to 8.0. In the anaerobic experiments the pH of the medium

after fermentation ranged from 5.0 to 6.0. As the fermentation of hay by the natural microflora was to be studied, no inoculations were made. Although no microbial analyses of the fermented hay were made, it was apparent that most of the samples had undergone decomposition by bacteria. A few of the samples showed evidence of decomposition by actinomycetes, and since their reactions were distinctly acid, the fermented plant materials were not subjected to chemical analyses.

In the first series of experiments two sets of cotton-stoppered flasks, one containing hay of the first cutting and the other hay of the third cutting, each with nutrient medium, were incubated for 30 days at 30° C. In this series each experiment was conducted in duplicate. In the second series the flasks containing the ground hay and nutrient medium were incubated first for 30 days at 30° and then for 30 days at 55°. In this series each experiment was conducted in triplicate. In the third series the flasks containing the ground hay and nutrient medium were each closed with a rubber stopper containing a mercury seal and were incubated under anaerobic conditions for 30 days at 30°. In this series the duplicate samples were combined and thoroughly mixed, and the composite sample was analyzed.

At the end of each incubation period in the first three series of experiments the residual plant material in each flask was filtered off, dried at 105° C., and weighed. From the weight of the material in each flask before and after fermentation the percentage loss due to microbial action was calculated. The composition of the residual plant material was then determined by the analytical methods previously described. By comparing these results with those obtained in the analysis of the materials in the original state, data were obtained showing the absolute loss of each constituent or group of constituents brought about by the action of the micro-organisms. The composition of the starting material is given in table 1. The results of the first, second, and third series of experiments are recorded in tables 2, 3, and 4, respectively.

TABLE 1.—*Composition of alfalfa used in microbiological decomposition experiments*[1]

[Percentage of ash was calculated on the basis of oven-dried material. All other results were calculated on the basis of oven-dried, ash-free material]

Constituents	First cutting		Third cutting	
	Percent	*Grams*	*Percent*	*Grams*
Ash	5.80		5.97	
Alcohol-benzene extractives	23.7	4.4650	27.3	5.1324
Cold-water extractives	10.8	2.0347	12.8	2.4064
Hot-water extractives	5.3	.9985	6.0	1.1280
Uronic acid anhydrides	11.6	2.1854	12.0	2.2560
Pentosans[2]	15.2	2.8636	11.6	2.1808
Cross and Bevan cellulose	30.9	5.8215	23.2	4.3614
Furfural in Cross and Bevan cellulose	13.8		12.5	
Cellulose	23.6	4.4462	18.2	3.4216
Lignin	9.7	1.8274	7.6	1.4288
Methoxyl in ash-free lignin	11.8		9.7	
Methoxyl in plant material	2.8	.5275	2.6	.4888
Methoxyl in extracted plant material	1.5	.2826	1.0	.1880
Crude protein, N×6.25	16.2	3.0520	21.3	4.0044

[1] 20 grams of dry material was used for each experiment.
[2] Corrected for furfural from uronic acids.

In the fourth series of experiments the course of the fermentation was followed by measuring the daily evolution of carbon dioxide from flasks containing hay and nutrients, and fermented under conditions similar to those of the second series—that is, for 30 days

at 30° C. and then for 30 days at 55°. Duplicate samples of hay from each cutting were prepared as described above, and the flasks were connected to the carbon dioxide absorbing apparatus described by Beavens and James (*2*). Daily titrations of the carbon dioxide evolved from the fermenting hay were made, and the results were recorded as milligrams of carbon.

RESULTS

For the sake of convenience, the results presented in each table will be dealt with separately. Table 1 shows that there was a decided difference in composition in the two cuttings of hay. This difference was reflected in the behavior of the two materials toward the action of micro-organisms as measured by the percentage loss of the various constituents and by the quantity of carbon dioxide evolved. Table 1 also shows that although the percentage of ash in the two cuttings of hay was about the same, the percentage of the other constituents was substantially different. In every case of the alcohol-benzene, cold and hot water extractives, uronic acid anhydrides, and crude protein (N×6.25) a greater percentage was found in the third-cutting than in the first-cutting alfalfa. However, in the pentosans, Cross and Bevan cellulose, furfural in Cross and Bevan cellulose, cellulose, lignin, methoxyl in lignin, and methoxyl in extracted and unextracted plant materials, the realtionship was reversed. The first-cutting alfalfa showed greater percentages of these constituents than the third-cutting alfalfa.

TABLE 2.—*Composition of alfalfa fermented for 30 days at 30° C. under aerobic conditions* [1]

FIRST CUTTING [2]

[All results were calculated on the basis of oven-dried, ash-free material]

Constituents	Sample 1			Sample 2		
	In alfalfa		Loss [3]	In alfalfa		Loss [3]
	Percent	*Grams*	*Percent*	*Percent*	*Grams*	*Percent*
Alcohol-benzene extractives	9.2	0.9236	79.3	9.1	0.8508	80.9
						[4] 80.1
Cold-water extractives	10.3	1.0341	49.1	11.5	1.0752	47.1
						[4] 48.1
Hot-water extractives	3.7	.3714	62.8	3.4	.3179	68.1
						[4] 65.4
Uronic acid anhydrides	7.4	.7429	66.0	7.0	.6545	70.0
						[4] 68.0
Pentosans [5]	15.5	1.5562	45.6	16.0	1.4960	47.7
						[4] 46.6
Cross and Bevan cellulose	43.0	4.3172	25.8	45.1	4.2168	27.5
						[4] 26.6
Furfural in Cross and Bevan cellulose	12.4			12.4		
				[4] 12.4		
Cellulose	33.9	3.4035	23.4	35.5	3.3192	25.3
						[4] 24.3
Lignin	17.0	1.7068	6.6	18.3	1.7110	6.3
						[4] 6.4
Methoxyl in ash-free lignin	14.1			13.9		
				[4] 14.0		
Methoxyl in plant material	3.4	.3413	35.3	4.1	.3833	27.3
						[4] 31.3
Methoxyl in extracted plant material	2.8	.2811	.5	3.0	.2805	.7
						[4] .6
Crude protein, N×6.25	12.8	1.2851	57.8	14.0	1.3090	57.1
						[4] 57.4

[1] 20 grams of dry material was used for each experiment. This corresponded to 18.84 and 18.80 g of ash-free material from the first and third cuttings, respectively.

[2] The weight of the dry, ash-free, fermented hay from the 2 samples at the end of the experiment was 10.04 and 9.35 g, respectively, which was 46.7 and 50.3 percent loss in weight, respectively.

[3] Calculated from data in table 1.

[4] The mean value of the 2 samples.

[5] Corrected for furfural from uronic acids.

TABLE 2.—*Composition of alfalfa fermented for 30 days at 30° C. under aerobic conditions*—Continued

THIRD CUTTING [6]

Constituents	Sample 1			Sample 2		
	In alfalfa		Loss [3]	In alfalfa		Loss [3]
	Percent	*Grams*	*Percent*	*Percent*	*Grams*	*Percent*
Alcohol-benzene extractives	14.1	1.2831	75.0	13.5	1.2366	75.9 [4] 75.4
Cold-water extractives	9.5	.8645	64.0	11.6	1.0625	55.8 [4] 59.9
Hot-water extractives	3.5	.3185	71.7	4.1	.3755	66.7 [4] 69.2
Uronic acid anhydrides	6.8	.6188	72.5	6.9	.6320	71.9 [4] 72.2
Pentosans [5]	12.6	1.1466	47.4	12.5	1.1450	47.4 [4] 47.4
Cross and Bevan cellulose	33.2	3.0212	30.7	33.2	3.0411	30.2 [4] 30.4
Furfural in Cross and Bevan cellulose	12.5			12.3 [4] 12.4		
Cellulose	26.1	2.3751	30.5	26.2	2.3999	29.8 [4] 30.1
Lignin	14.9	1.3559	5.1	14.5	1.3282	7.0 [4] 6.0
Methoxyl in ash-free lignin	11.2			10.6 [4] 10.9		
Methoxyl in plant material	3.0	.2730	44.1	2.8	.2564	47.5 [4] 45.8
Methoxyl in extracted plant material	2.0	.1820	3.2	1.9	.1740	7.4 [4] 5.3
Crude protein, N×6.25	18.6	1.6926	57.7	19.0	1.7404	56.5 [4] 57.1

[3] Calculated from data in table 1.
[4] The mean value of the 2 samples.
[5] Corrected for furfural from uronic acids
[6] The weight of the dry, ash-free, fermented hay from the 2 samples at the end of the experiment was 9.10 and 9.16 g, respectively, which was 51.6 and 51.2 percent loss in weight, respectively.

The results of microbial decomposition of alfalfa hay under aerobic conditions when allowed to proceed for 30 days at 30° C. is shown in table 2. It will be observed that the total decomposition as measured by the percentage loss in weight was slightly less in the first-cutting alfalfa than in the third-cutting alfalfa. The alcohol-benzene extractives showed a somewhat greater loss in the first-cutting than in the third-cutting material. However, in the cold- and hot-water extractives and uronic acid anhydrides this relationship was reversed, although the differences were not large. The percentage loss of pentosans and crude protein (N×6.25) in the two plant materials was about the same. The Cross and Bevan cellulose, cellulose, and methoxyl in the plant material (unextracted) showed a greater loss in the third-cutting than in the first-cutting material. The percentage of furfural in the Cross and Bevan cellulose of the first-cutting material decreased somewhat, although in the third-cutting material no such effect was noted. The loss of lignin in the plant materials of both cuttings was of the same general order of magnitude. In the third-cutting material the loss of lignin is indicated not only in the results obtained by direct analysis for lignin but also by the loss of methoxyl in the extracted plant material. There is no explanation for the discrepancy between the loss of lignin in the first-cutting material and the small loss in the methoxyl in extracted plant material. In the main, the results indicate that under the conditions of this series of experiments the lignin fraction was the most resistant to microbial attack, although it did suffer some decomposition.

TABLE 3.—*Composition of alfalfa fermented for 30 days at 30° C. and for 30 days at 55° C. under aerobic conditions* [1]

[All results calculated on the basis of oven-dried, ash-free material]

FIRST CUTTING [2]

Constituents	Sample 1			Sample 2			Sample 3		
	In alfalfa		Loss [3]	In alfalfa		Loss [3]	In alfalfa		Loss [3]
	Percent	*Grams*	*Percent*	*Percent*	*Grams*	*Percent*	*Percent*	*Grams*	*Percent*
Alcohol-benzene extractives	15.6	1.2292	72.4	13.4	1.2381	72.2	14.9	1.3007	70.8 [4] 71.8
Cold-water extractives	8.0	.6304	69.0	7.0	.6468	68.2	8.7	.7595	62.6 [4] 66.6
Hot-water extractives	3.1	.2442	75.5	3.2	.2956	70.4	3.4	.2968	70.2 [4] 72.0
Uronic acid anhydrides	6.8	.5358	75.4	6.1	.5636	74.2	6.2	.5412	75.2 [4] 74.9
Pentosans [5]	16.4	1.2923	54.8	15.7	1.4506	49.3	15.0	1.3095	54.2 [4] 52.7
Cross and Bevan cellulose	36.8	2.8998	50.1	43.4	4.0101	31.1	38.3	3.3435	42.5 [4] 41.2
Furfural in Cross and Bevan cellulose	13.5			13.0			13.4 [4] 13.3		
Cellulose	28.3	2.2300	49.8	33.8	3.1231	29.7	29.5	2.5753	42.0 [4] 40.5
Lignin	19.2	1.5129	17.2	19.1	1.7648	3.4	18.7	1.6325	10.6 [4] 10.4
Methoxyl in ash-free lignin	11.3			12.0			10.7 [4] 11.3		
Methoxyl in plant material	3.4	.2679	49.2	4.2	.3880	26.4	4.0	.3492	33.8 [4] 36.4
Methoxyl in extracted plant material	2.6	.2048	27.5	2.7	.2494	11.7	2.6	.2269	19.7 [4] 19.6
Crude protein, N×6.25	13.7	1.0795	64.6	10.8	.9979	67.3	12.2	1.0650	65.1 [4] 65.6

THIRD CUTTING [6]

Constituents	Sample 1			Sample 2			Sample 3		
	In alfalfa		Loss [3]	In alfalfa		Loss [3]	In alfalfa		Loss [3]
	Percent	*Grams*	*Percent*	*Percent*	*Grams*	*Percent*	*Percent*	*Grams*	*Percent*
Alcohol-benzene extractives	20.4	1.8686	63.5	23.0	1.6744	67.3	17.2	1.4396	71.9 [4] 67.6
Cold-water extractives	10.5	.9618	60.0	11.8	.8590	64.3	7.3	.6110	74.6 [4] 66.3
Hot-water extractives	2.8	.2564	77.2	3.8	.2766	75.4	2.5	.2092	81.4 [4] 78.0
Uronic acid anhydrides	6.7	.6137	72.7	7.3	.5314	76.4	5.6	.4687	79.2 [4] 76.1
Pentosans [5]	12.0	1.0992	49.6	12.3	.8954	58.9	12.0	1.0044	53.9 [4] 54.1
Cross and Bevan cellulose	34.8	3.1876	26.9	36.0	2.6208	39.9	32.6	2.7286	37.4 [4] 34.7
Furfural in Cross and Bevan cellulose	12.4			12.5			12.4 [4] 12.4		
Cellulose	27.4	2.5098	26.6	28.3	2.0602	39.7	25.7	2.1510	37.1 [4] 34.4
Lignin	14.7	1.3465	5.7	15.0	1.0920	23.5	13.5	1.1299	20.9 [4] 16.7
Methoxyl in ash-free lignin	9.1			9.9			10.1 [4] 9.7		
Methoxyl in plant material	2.7	.2473	49.4	2.7	.1965	59.8	2.4	.2008	58.9 [4] 56.0
Methoxyl in extracted plant material	2.0	.1832	2.5	2.0	.1456	22.5	1.9	.1590	15.4 [4] 13.4
Crude protein, N×6.25	13.9	1.2732	68.2	19.8	1.4414	64.0	12.8	1.0713	73.2 [4] 68.4

[1] 20 g of dry material was used for each experiment. This corresponded to 18.84 and 18.80 g of ash-free material from the first and third cuttings, respectively.

[2] The weight of dry, ash-free, fermented hay from the 3 samples at the end of the experiment was 7.88, 9.24, and 8.73 g, respectively, which was 58.1, 50.9, and 53.6 percent loss in weight, respectively.

[3] Calculated from data in table 1.

[4] The mean value of the 3 samples.

[5] Corrected for furfural from uronic acids.

[6] The weight of the dry, ash-free, fermented hay from the 3 samples at the end of the experiment was 9.16, 7.28, and 8.37 g, respectively, which was 51.2, 61.2, and 55.5 percent loss in weight, respectively.

In table 3 figures are presented showing the composition and percentage loss suffered by the several constituents of alfalfa hay when fermented under aerobic conditions, first for 30 days at 30° C. and then for an additional 30 days at 55°. In this series of experiments, the results indicate the combined effect of the decomposition by mesophilic and thermophilic micro-organisms and the longer incubation period. It will be observed that, as in the previous series of experiments (table 2), the total decomposition as measured by the average percentage loss of the organic fraction of the plant materials was slightly greater in the third-cutting than in the first-cutting alfalfa. The alcohol-benzene extractives showed a greater loss in the first-cutting than in the third-cutting material, as was also the case in the previous series of experiments (table 2). The average loss of cold-water extractives was about the same in both cuttings. It was, however, considerably greater than when the plant materials were fermented for 30 days at 30° (table 2). In general, this was the case with all the other constituents except the alcohol-benzene extractives. The hot-water extractives, uronic acid anhydrides, pentosans, methoxyl in plant material (unextracted), and crude protein ($N \times 6.25$) suffered a somewhat greater loss in the third-cutting than in the first-cutting material. In the Cross and Bevan cellulose and the cellulose proper, the reverse was true; that is, the first-cutting material suffered the greater percentage loss. The percentage of furfural in the Cross and Bevan cellulose remained substantially the same as in the original plant materials, thus indicating that the micro-organisms did not preferentially attack the xylan fraction of the Cross and Bevan cellulose. Apparently the "pure" cellulose fraction and the xylan fraction of the Cross and Bevan cellulose were attacked at approximately the same rate. The principal difference between this series of experiments and the previous one lies in the results obtained on the microbiological decomposition of lignin. In the main, the lignin from both the first and third cuttings of alfalfa suffered a greater loss than in the previous series of experiments (table 2). The results are rather irregular, but this was evidently not caused by any error in the analytical procedure, as the results on the loss of methoxyl in the extracted plant material show a similar tendency. In working with mixed cultures of organisms it is, of course, difficult to make experimental conditions identical. Under optimum conditions, the organisms capable of breaking down lignin themselves or in symbiosis with other organisms become more active, and there is accordingly a correspondingly greater loss of lignin than when conditions are not most suitable for their activity. The percentage of methoxyl in the residual lignin of this series was about the same as in the lignin from the original materials.

TABLE 4.—*Composition of alfalfa fermented for 30 days at 30° C. under anaerobic conditions* [1]

[All results calculated on the basis of oven-dried ash-free material]

Constituents	First cutting [2]			Third cutting [2]		
	In alfalfa		Loss [3]	In alfalfa		Loss [3]
	Percent	*Grams*	*Percent*	*Percent*	*Grams*	*Percent*
Alcohol-benzene extractives	9.0	1.0197	77.1	11.6	1.0718	79.1
Cold-water extractives	4.9	.5551	72.7	5.3	.4897	79.6
Hot-water extractives	3.6	.4078	59.1	3.7	.3418	69.7
Uronic acid anhydrides	8.4	.9517	56.4	8.2	.7576	66.4
Pentosans [4]	17.3	1.9600	31.5	15.2	1.4044	35.6
Cross and Bevan cellulose	46.4	5.2571	9.7	40.8	3.7699	13.5
Furfural in Cross and Bevan cellulose	12.6			12.0		
Cellulose	36.4	4.1241	7.2	32.4	2.9973	12.4
Lignin	14.5	1.6428	10.1	14.2	1.3120	8.1
Methoxyl in ash-free lignin	13.4			10.0		
Methoxyl in plant material	3.1	.3512	33.4	2.5	.2310	52.7
Methoxyl in extracted plant material	2.2	.2492	11.8	1.9	.1755	6.6
Crude protein, N×6.25	14.2	1.6088	47.2	17.2	1.5892	60.3

[1] 20 grams of dry material was used for each experiment. This corresponded to 18.84 and 18.80 g of ash-free material from the first and third cuttings, respectively.
[2] The weight of dry, ash-free, fermented hay from the first and third cuttings was 11.33 and 9.24 g, respectively, which was 39.8 and 50.8 percent loss in weight, respectively.
[3] Calculated from data in table 1.
[4] Corrected for furfural from uronic acids.

In table 4 results are presented showing the effect when first and third cuttings of alfalfa are fermented for 30 days at 30° C. under anaerobic conditions. In this series of experiments also there was a greater loss in weight in the third-cutting material than in that from the first cutting. The alcohol-benzene extractives, cold- and hot-water extractives, uronic acid anhydrides, pentosans, Cross and Bevan cellulose, cellulose, methoxyl in plant material (unextracted), and crude protein (N × 6.25) suffered a greater loss in the third-cutting than in the first-cutting material. The furfural-yielding fraction of the Cross and Bevan cellulose was, in part, decomposed. The Cross and Bevan cellulose fraction, it will be noted, decomposed less under anaerobic conditions than under aerobic conditions. What came as a surprise, however, was that the lignin in the alfalfa from both cuttings was decomposed as greatly under anaerobic conditions as under aerobic conditions. It is generally believed that lignin is rather resistant to microbial attack under anaerobic conditions. The results, however, are in agreement with the recent findings of Boruff and Buswell (*4*), who reported a loss of lignin in corncobs that had been fermented anaerobically. The results on the loss of methoxyl in the extracted plant material in the main support the results on the loss of lignin obtained by direct analysis for lignin. As compared with the other constituents, with the possible exception of the cellulose, the lignin proved to be the most resistant to microbial attack. As in table 2, the methoxyl in the residual lignin was also somewhat greater than in the lignin from the original plant materials.

The results of the fourth series of experiments are shown graphically in figures 1 and 2. The samples were incubated for 30 days at 30° C. and then for an additional 30 days at 55°. The total quantities of carbon dioxide evolved (plotted as milligrams of carbon) during the aerobic decomposition are shown in figure 1. During both incubation periods (first period, 30 days at 30°; second period,

30 days at 55°) a greater loss of carbon (as carbon dioxide) occurred in the third-cutting material than in the first-cutting material. During the first few days of the first incubation period, the difference in the quantities of carbon dioxide evolved from both the first- and third-cutting hay was greater than in the remainder of the test period. Although in the second incubation period the third-cutting material showed a more active decomposition than the first-cutting material, it is apparent from figure 1 that the two curves have a tendency to converge near the end of the experimental period.

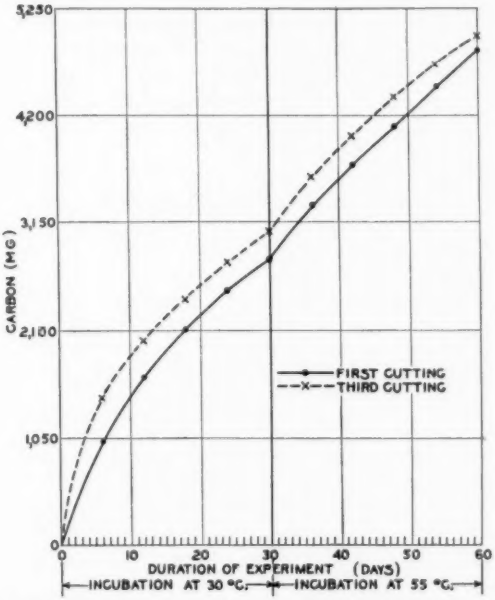

FIGURE 1.—Total carbon dioxide (calculated as carbon) evolved during the microbial decomposition of two cuttings of alfalfa hay under aerobic conditions. (Twenty grams of hay was used in each experiment.)

The results of the daily titrations of carbon dioxide evolved (plotted as milligrams of carbon) during the aerobic decomposition of the two cuttings of alfalfa hay are shown graphically in figure 2. It will be observed that the greatest rate of decomposition occurred during the first 4 to 5 days of the first incubation period (30 days at 30° C.), after which it gradually decreased. During the second incubation period (30 days at 55°) another definite increase occurred in the decomposition of both cuttings of hay, although it was not so great as in the first period of incubation. This was evidently caused by a change from the mesophilic type of microflora to the thermophilic type and a speeding up of their metabolic activities, resulting in increased decomposition. Consideration has been given to the fact that an increase in temperature of a carbon dioxide solution from 30° to 55° will, of course, cause some liberation of this gas, although one would expect this to occur shortly after the rise in temperature.

However, as is shown in figure 2, the maximum quantity of carbon dioxide was not evolved until the third day of the second period of fermentation, so that, in the main, the increased evolution of carbon dioxide may be considered as caused by increased microbial activity.

DISCUSSION

Investigators of spontaneous ignition of hay and similar agricultural materials now recognize that micro-organisms play an important role in causing the initial rise in temperature of the fermenting plant material (*7*, *18*). However, the rise in temperature from about 70° to 80° C., when all microbial activity has ceased, to the ignition temperature of the plant material has been the subject of much speculation. No attempt will be made here to review

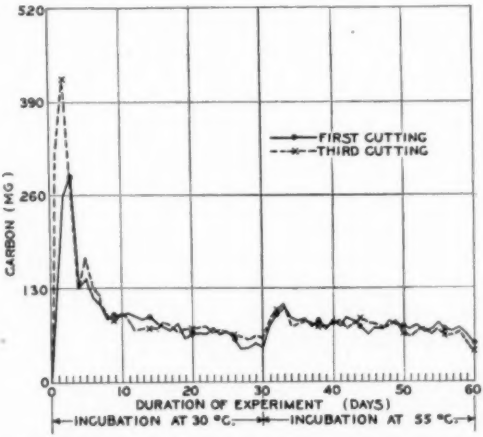

FIGURE 2.—Carbon dioxide (calculated as carbon) evolved daily during the microbial decomposition of two cuttings of alfalfa hay. (Twenty grams of hay was used in each experiment.)

the various hypotheses proposed to account for this phenomenon,[6] as this has already been done by Browne (*5*, *6*), who put forward the hypothesis that the rise in temperature is brought about by the oxidation of unsaturated compounds produced as a result of the dehydroxylation of the carbohydrates by micro-organisms. While the oxidation of such unsaturated degradation products from carbohydrates may account for the rise in temperature of the vegetable material, attention is called to another class of unsaturated compounds, namely, the lignins, that may play an important role in bringing about the spontaneous ignition of plant materials. From tables 2, 3, and 4 it will be observed that because of the more rapid decomposition of the other major plant constituents, such as the carbohydrates, fats, proteins, and polyuronides, there is a large proportion of lignin in the residual plant material. Isolated lignin, that is, lignin prepared in the laboratory, is partly unsaturated, as

[6] After the present paper had been prepared for publication an article by Rudge (*28*) appeared, suggesting that spontaneous combustion may be caused entirely by chemical action. He assumes that cellulose reacts with bicarbonates to form a condensation product having a composition analogous to that of cellulose xanthate. It is suggested that the cellulose-bicarbonate condensation product may undergo rapid oxidation, resulting under favorable circumstances in the attainment of the ignition point.

is evident from the fact that it will absorb iodine from an iodine solution. It is conceivable that lignin freed from its combination with the carbohydrates by microbial activity may be even more unsaturated, as it is reasonable to suppose that lignin liberated under comparatively mild conditions would be less polymerized. Under suitable conditions this free lignin may readily absorb oxygen, with the evolution of heat. This rise in temperature will cause a degradation of the lignin such as is produced when this substance is subjected to dry distillation. Under these conditions lignin produces, in addition to other substances, a whole series of polyhydroxy phenols (*23*). These phenols are related to catechol and pyrogallol, which under suitable conditions absorb oxygen with avidity. This will bring about a further rise in temperature until the ignition point of the plant material is reached.

SUMMARY

A study was made of the microbiological decomposition of first- and third-cutting alfalfa hay under aerobic conditions at incubation temperatures of 30° and 55° C. and under anaerobic conditions at 30°. The third-cutting material decomposed somewhat more rapidly than the first-cutting material. The greatest decomposition took place in the alcohol-benzene extractives, the hot- and cold-water extractives, the uronic acid anhydrides (the pectins and the polyuronide fraction generally), the crude protein (N×6.25), and the methoxyl in the plant material (unextracted). The pentosans decomposed somewhat more slowly, and the rate of decomposition of the Cross and Bevan cellulose and of the "pure" cellulose was still less. In general, the lignin was the most resistant of all the major plant constituents, although substantial losses of lignin occurred under aerobic and anaerobic conditions. The possible role of lignin in the spontaneous ignition of hay is discussed.

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SOME FACTORS INFLUENCING THE YIELD AND MORTALITY OF PONDEROSA PINE IN THE SOUTHWEST [1]

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INTRODUCTION

A knowledge of those factors influencing the yield of timber stands that are more or less subject to control by man is of practical importance to the forester. The following analysis of the effect on yield of the factors of volume left after cutting and the average size of reserved trees was undertaken in an effort to add to that knowledge. Data for such a study were available from permanent sample plots established during the past 20 years by the Southwestern Forest and Range Experiment Station on cut-over areas in the ponderosa pine type for the purpose of determining the possible yield of saw timber at the end of a cutting cycle. Analyses of these plots were made by Krauch in 1926 [2] with particular emphasis on the individual tree. In the present study, unit of area (1 acre) has been substituted as the basis of analysis, a method that lends itself readily to statistical treatment.

Because analyses of the relation of increment to time based on periods of 20 years and less usually lead to dangerous extrapolation and are of little practical assistance in predicting yield, this relation was not considered except in a preliminary fashion, and the analysis was centered on the yield at the end of 15 years, in which sense time could be considered as held constant.

MATERIAL AND METHODS

Ninety-three 1-acre plots on which growth records had been taken over a period of at least 15 years were used as the basis of the study. All plots were established immediately after logging, and hence represented the total change subsequent to cutting. There is a rather wide diversity of soil conditions among the plots, but no claim is made of exact representativeness of the universe of cut-over ponderosa pine (*Pinus ponderosa* Lawson).

For each 1-acre plot the following values were determined: (1) Volume of reserved stand; (2) volume of the average tree; (3) increment at the end of 15 years; and (4) total mortality by 5-year periods. All quantities are given in Scribner board feet, the merchantability limits being 10 inches d. b. h.[3] and an 8-inch top diameter.

The reserved volume on each plot was determined by the use of volume tables. In order to eliminate so far as possible any biased

[1] Received for publication Jan. 29, 1935; issued July 1935.

[2] KRAUCH, H. THE DETERMINATION OF INCREMENT IN CUT-OVER STANDS OF WESTERN YELLOW PINE IN ARIZONA. Jour. Agr. Research 32: 501–541, illus. 1926.

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[3] Diameter breast high, or 4½ feet above the ground.

errors and to keep the random errors as small as possible, separate volume tables were used for those plots representing two widely different soil types. Separate tables were used also for the two broad age classes, yellow pine and blackjack.[4] Trees that died during the period after cutting were removed from the reserved volume in order to prevent excessive variation in yield and are treated separately under Mortality Analysis (p. 783).

The volume of the average tree on each acre was determined by dividing the total reserved volume by the number of merchantable trees. This variable was selected because it gave some indication of the size, and roughly of the age, of the reserved stand left on each plot.

The increment was found by simply subtracting the original volume of the reserved stand from the volume at the end of 15 years. No allowance was made for a possible change in form of the reserved trees after cutting, although such a change creates a small biased error in the increment values.

In the following analysis advantage was taken of correlation methods and of some of the analytical systems developed by Fisher.[5] The influence of random errors in the independent and dependent variables on a few of the more important statistical constants was kept in mind. It has been demonstrated that random errors in the dependent variable lower the correlation coefficient but do not appreciably alter the regression coefficients, while random errors present only in the independent variables and those in both dependent and independent variables, tend to decrease the magnitude of both the correlation and regression coefficients.[6] It is obvious that in analyses of this kind any tests of significance between regression and correlation coefficients are of questionable value, since it cannot be definitely stated whether the differences observed are due to some definite assignable cause or to the inability to measure each variable accurately. This should not, however, vitiate the test of whether a correlation or regression coefficient is significant from zero, because higher values for both could reasonably be expected if it were possible to measure the variables exactly. In other words, if either coefficient is found to be significant when random errors are present, enough evidence must be accumulated within the data to indicate it over and above the influence produced by random errors.

YIELD ANALYSIS

As a preliminary step, the gross correlation coefficients for increment and reserved volume, for increment and volume of the average tree, and for reserved volume and volume of the average tree were determined and are given in table 1. The symbols used in the discussion are defined as follows:

X_1 = increment at the end of 15 years.
X_2 = reserved volume.
X_3 = volume of average tree on plot.

[4] "Yellow pines" are ponderosa pines 200 years old and older; they have a deep-yellowish or cinnamon-brown bark. "Blackjacks" are 150 years old or less and have dark, nearly black bark.

[5] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 3, 283 pp., illus. Edinburgh and London. 1930.

[6] EZEKIEL, M. METHODS OF CORRELATION ANALYSIS. 427 pp., illus. New York. 1930. (Ch. 19.)

TABLE 1.—*Correlation coefficients for increment and reserved volume, increment and volume of average tree, and reserved volume and volume of average tree*

Variables	Gross coefficient of correlation	Gross coefficient of determination	Significance [1]
X_1 and X_2	0.7982	0.6371	Significant.
X_1 and X_3	−.3546	.1258	Do.
X_2 and X_3	−.0067		Insignificant.

[1] Determined by the aid of Fisher's table V. A., p. 176. (See footnote 5.)

The coefficients of determination, which are equal to the coefficient of correlation squared, are included because they express much more clearly the relationship between variables.

From table 1 it is evident that both reserved volume and the volume of the average tree are important factors influencing increment. Lack of intercorrelation between these factors indicates that a heavy reserved stand is not necessarily composed of large trees nor is a light reserved stand necessarily composed of small trees. As a matter of fact, the lack of significant correlation implies that the exact composition of each plot is due to chance. This lack of intercorrelation further suggested the possibility of improving the coefficient of total determination to approximately the sum of both gross determinations given in table 1, by combining the two variables X_2 and X_3 into a multiple correlation.

When this suggestion was followed out, the resulting coefficient of total determination was 0.7591 and the corresponding correlation coefficient 0.8712. Other important points in this analysis are given in table 2.

TABLE 2.—*Contribution of reserved volume and volume of average tree to volume growth*

Variables	Net regression coefficient	Standard error / regression coefficient ×100	Coefficient of separate determination
X_1 and X_2	0.2486±0.0162	6.5	0.6352
X_1 and X_3	.9377± .1389	14.8	.1239
Total			.7591

The resulting regression equation is

$$X_1 = 647.43 + 0.2486X_2 - 0.9377X_3.$$

From table 2, column 4, it can be seen that the two coefficients of separate determination are approximately equal to the corresponding gross determination coefficients in table 1. The standard errors of the two regression coefficients are included in column 2, and in column 3 they are expressed as a percentage of the corresponding regression coefficients. It is evident that reserved volume measures its influence on increment rather accurately and that the

volume of the average tree also measures its influence on increment, but to a lesser degree of reliability.

The net regression coefficient of X_1 on X_3 is negative, which means that for a given reserved volume the yield per acre tends to become

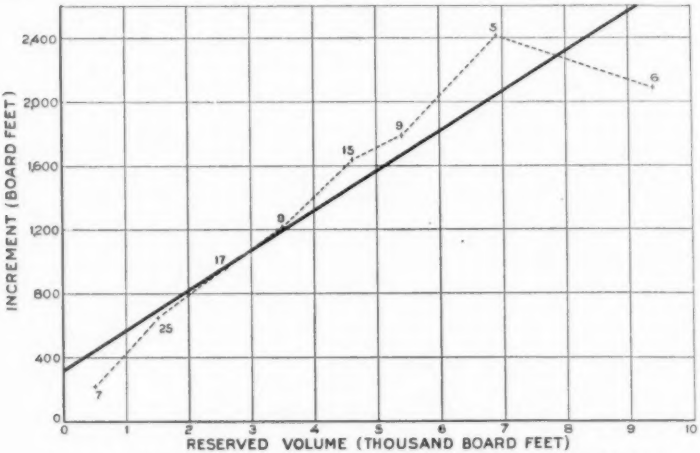

FIGURE 1.—Net regression line of increment over reserved volume with residuals.

less as the volume of the average tree increases. In other words, so far as increment is concerned it would be much more desirable to have the reserved volume divided among a relatively large number of small trees than among a few large ones.

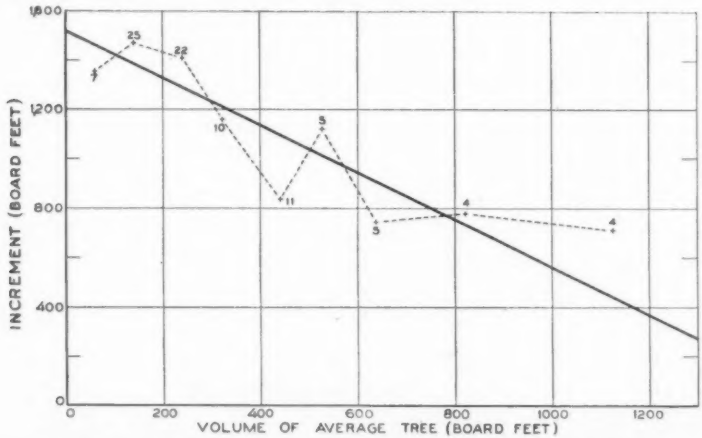

FIGURE 2.—Net regression line of increment over volume of average tree with residuals.

Up to this point the analysis has assumed that the influence of X_2 and X_3 on increment was linear. The truth of this assumption can be tested by plotting the residuals over the net regression lines

oi increment over reserved volume, and increment over the volume of the average tree. The results of these calculations are shown graphically in figures 1 and 2. There is a marked indication of

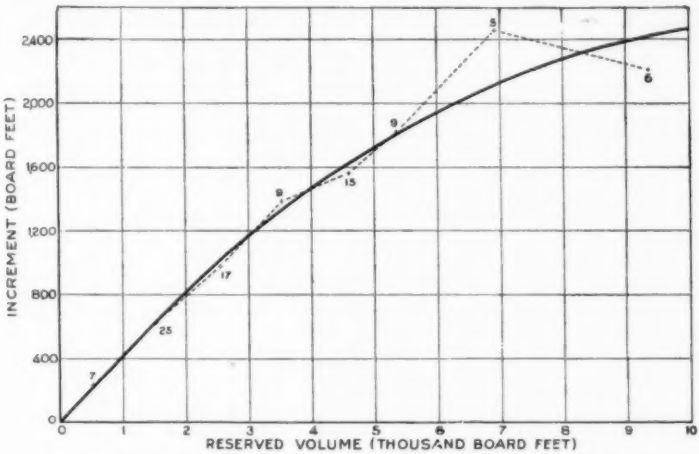

FIGURE 3.—Final net regression curve of increment over reserved volume with residuals.

curvature in figure 1 which is evident also, though it is not so pronounced, in figure 2.

By introducing curvature into the regression surface, it should be possible to further improve the coefficient of total determina-

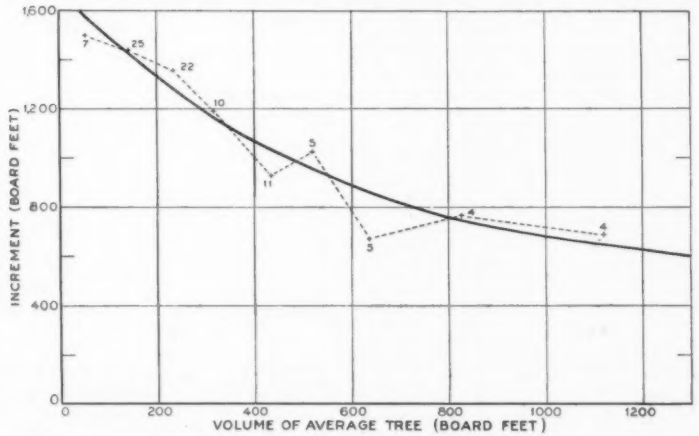

FIGURE 4.—Final net regression curve of increment over volume of average tree with residuals.

tion. This was done by the method of successive approximation.[7] The final net regression curves are given in figures 3 and 4. They represent graphically the influence of reserved volume of increment

[7] EZEKIEL, M. See ch. 14 of citation mentioned in footnote 6.

with the volume of the average tree held constant at its mean (351 board feet) and the influence of the volume of the average tree on increment with the reserved volume held constant at its mean (3,490 board feet). From figure 3 it is apparent that increment increases constantly with an increase in reserved volume but at a constantly decreasing rate. In figure 4 this relationship is reversed, that is, the increment becomes constantly less with an increase in the volume of the average tree, but the decrease comes at a constantly decreasing rate.

A test for joint correlation of the type where $X_1=\theta[a+f(X_2)-f(X_3)]$ was made. The results are shown graphically in figure 5. Since

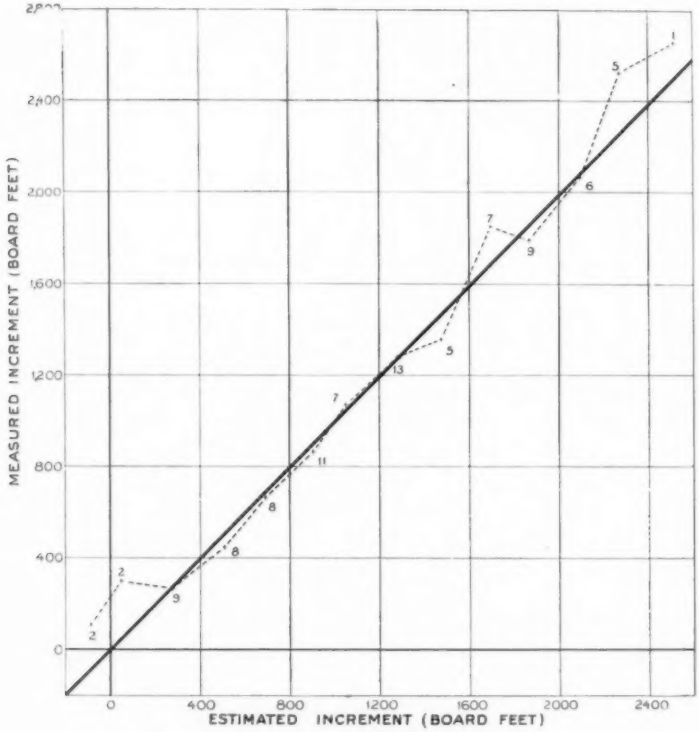

FIGURE 5.—Relation of measured to estimated increment

there was no marked indication of curvature present, it was concluded that this type of joint relationship did not exist. Further tests for joint correlation could not be made because of insufficiency of data.

Through the introduction of curvature in the regression surface it was possible to improve the correlation coefficient from 0.8712 to 0.8974 and the corresponding coefficient of determination from 0.7591 to 0.8054. From this it was concluded that approximately 80 percent of the variance of increment at the end of 15 years is associated with reserved volume and the volume of the average tree left after cutting.

MORTALITY ANALYSIS

Any determination of yield for selectively cut stands is more or less affected by loss through mortality. The amount to be deducted from gross yields due to this factor alone is subject to considerable error because of its extreme variability. Available data now indicate that if mortality continues at its present rate the annual loss from this source will be between 10 and 30 percent of the average annual growth. It has, however, been assumed that this loss will decrease with time after cutting, and table 3, which gives the mortality for each plot by 5-year periods and the mean mortality for all plots in these periods, appears to lend credence to this hypothesis.

TABLE 3.—*Loss in board feet through mortality, by 5-year periods*

Plot no [1]	0–5 years	6–10 years	11–15 years	Total loss	Mean period loss
	Board feet	*Board feet*	*Board feet*	*Board feet*	*Board feet*
1	821	0	0	821	273. 67
2	580	28	0	608	202. 67
5	0	29	0	29	9. 67
7	1, 380	1, 196	655	3, 231	1, 077. 00
8	57	50	70	177	59. 00
9	0	0	39	39	13. 00
11	0	900	0	900	300. 00
12	855	290	1, 933	3, 078	1, 026. 00
13	0	1, 180	0	1, 180	393. 33
15	1, 260	0	0	1, 260	420. 00
17	0	1, 175	0	1, 175	391. 67
19	32	0	0	32	10. 67
20	29	389	31	449	149. 67
23	0	42	0	42	14. 00
25	0	31	0	31	10. 33
26	0	0	37	37	12. 33
28	632	28	0	660	220. 00
29	840	0	0	840	280. 00
31	34	902	0	936	312. 00
32	0	277	0	277	92. 33
35	0	37	0	37	12. 33
37	0	0	1, 370	1, 370	456. 67
40	1, 395	0	0	1, 395	465. 00
42	755	0	0	755	251. 67
46	778	0	0	778	259. 33
47	615	0	47	662	220. 67
50	0	42	0	42	14. 00
52	0	0	692	692	230. 67
54	0	0	645	645	215. 00
55	0	1, 280	0	1, 280	426. 67
58	459	0	0	459	153. 00
59	106	0	0	106	35. 33
61	603	985	0	1, 588	529. 33
62	0	0	1, 520	1, 520	506. 67
64	0	0	25	25	8. 33
67	21	0	0	21	7. 00
68	0	0	512	512	170. 67
70	66	157	0	223	74. 33
71	0	0	88	88	29. 33
74	29	0	132	161	53. 67
76	0	194	305	499	166. 33
78	0	227	0	227	75. 67
79	58	0	0	58	19. 33
82	0	27	0	27	9. 00
84	0	0	160	160	53. 33
86	94	0	0	94	31. 33
87	0	0	52	52	17. 33
89	0	618	0	618	206. 00
91	0	449	0	449	149. 67
Total	11, 499	10, 533	8, 313	30, 345	10, 115. 00
Mean	123. 6452	113. 2581	89. 3871	326. 29032	108. 76344

[1] Those of the 93 plots on which no trees died during the 15-year period are not listed

The hypothesis can be tested for the early period after cutting by the method of analysis of variance. Table 4 summarizes the analysis of the data given in table 3, the sum of squares within periods being equal to $\overset{kn}{\underset{1}{S}}(x-\bar{x}_p)^2$.

TABLE 4.—*Summary of analysis of mortality data in table 3*

Description	Degrees of freedom	Sum of squares	Mean square	Standard deviation	Natural log of standard deviation
Within periods	276	26, 214, 449. 16	94, 979. 89	308. 188	5. 7307
Between periods	2	57, 391. 28	28, 695. 64	169. 398	5. 1323
Total	278	26, 271, 840. 44	94, 503. 02	307. 413	[1] 0. 5984

[1] The value of z, determined as insignificant by the aid of Fisher's table 6. (See footnote 5.)

Where

k=number of plots used in a period
x=mortality
$\bar{x}_p$=mean mortality for period
n=number of periods

the sum of the squares between periods equals

$$k\overset{n}{\underset{1}{S}}(\bar{x}_p-\bar{x})^2$$

and

$\bar{x}$=the general mean for all plots.

The lack of significant intraclass correlation indicates that not enough evidence has been accumulated to justify the conclusion that mortality is related to time since cutting. Although the mean mortality by periods shows a progressive decrease, the differences between means can readily be explained as being caused by errors of random sampling and not necessarily by some influence of time.

The foregoing analysis also indicates that the best estimate of the standard deviation of mortality is 307.413. With the standard deviation of mortality known, it is possible to determine the standard error of the mean from the relationship

$$s_m=\frac{s}{\sqrt{n}}$$

where

s=estimated standard deviation
s_m=estimated standard error or mean
n=number of observations.

Substituting the observed figures—

$$\frac{307.413}{\sqrt{279}}=18.40.$$

This establishes the standard error of the mean mortality as 18.40 board feet.

The average growth for a period of 5 years is approximately 300 board feet. Should it be desired to determine the average periodic

mortality within an accuracy of ±5 percent of the periodic growth and with odds 19 to 20 that the true mortality will be within this range, the area which must be sampled to assure this reliability can be determined from the formula—

$$n=\left(\frac{s^2}{s_m}\right)$$

where n=number of acres
s=the best estimated standard deviation of population in question
s_m=desired standard error.

Substituting and solving—

$$n=\left(\frac{307.413}{7.5}\right)^2=1{,}680 \text{ acres.}$$

For units smaller than 1,680 acres it is necessary to cover the area 100 percent to insure the above-stated accuracy of the mean. For larger units the percentage of area to be covered naturally becomes less.

It is sometimes possible to reduce the magnitude of the standard error of a mean by satisfactorily establishing correlation within the sample. In this case it appeared probable that mortality at the end of 15 years might conceivably be related to the amount of reserved volume and the volume of the average tree. A multiple correlation was performed, using the above-mentioned factors as independent variables, and a correlation coefficient of 0.2922 was obtained. The corresponding determination coefficient was 0.0854, which implies that only 8.5 percent of the variance of mortality is associated with the amount of reserved volume and the volume of the average tree.

The coefficient of multiple correlation in this case was so low that it appeared advisable to test it for significance. A summary of this test is given in table 5.

TABLE 5.—*Summary of test for significance of coefficient of multiple correlation between mortality at 15 years, amount of reserved volume, and volume of average tree*

Description	Degree of freedom	Sum of squares	Mean squares	One-half natural log
Regression formula	3	2,787,094.99	929,031.66	6.87095
Deviation from regression	90	29,855,077.88	331,723.09	6.35603
Value of z				[1] 0.51492

[1] Determined as significant by the same test used for z in table 4.

Since the value of z was found to be significant it was concluded that reserved volume and the volume of the average tree left after cutting have some influence on mortality.

The reliability of each regression coefficient was also analyzed, and a summary of the results is given in table 6. It is evident that, while the reserved volume and the volume of the average tree are significantly related to mortality, they do not measure this relation accurately, since in both cases the standard errors are equal to approximately one-half of the corresponding regression coefficients.

In figures 6 and 7 the net regression lines of mortality over reserved volume and mortality over the volume of the average tree are plotted with the corresponding residuals. It is clear from this that an increase

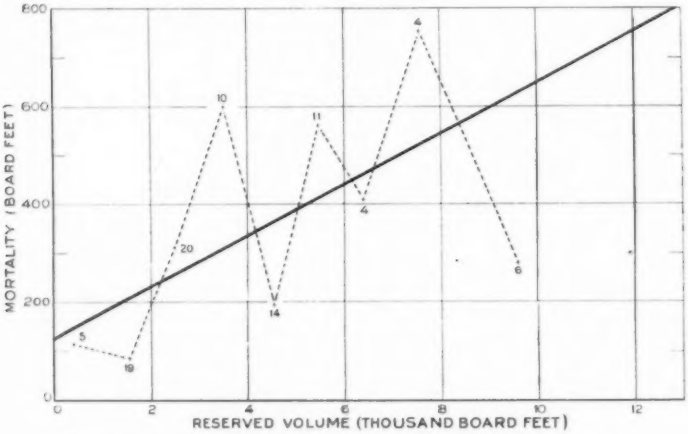

FIGURE 6.—Net regression line of mortality over reserved volume with residuals.

in either reserved volume or the size of the average tree leads to greater mortality. Casual observation of the plotted data alone indicates that this relationship is not very strong, and although a straight

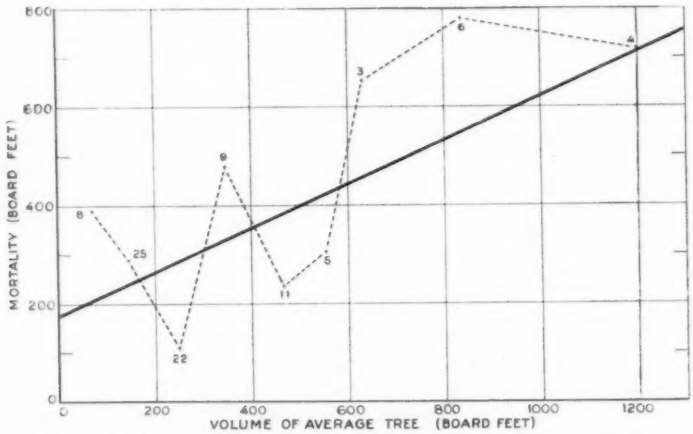

FIGURE 7.—Net regression line of mortality over volume of average tree with residuals.

line has been fitted to the data, it cannot be definitely assumed that the relationship is proportional.

TABLE 6.—*Summary of test for reliability of regression coefficients of correlation between mortality at 15 years, amount of reserved volume, and volume of average tree*

Variables	Net regression coefficients	Standard error	Regression coefficient / Standard error	Standard error / Regression coefficient ×100
X_1 and X_2	0.0530	0.0258	2.0542	48.7
X_1 and X_3	.4500	.2239	2.0099	49.8

SUMMARY

Analysis of factors influencing yield of timber stands indicates that the increment per acre at the end of 15 years after cutting is closely related to the volume of the reserved stand and the volume of the average tree. These two variables alone explain approximately 80 percent of the variance of increment; the remaining 20 percent is attributed to such factors as the degree of release, site differences, and differences in vigor of reserved stands.

By holding constant the influence of the size of trees left after cutting, it was found that the yield increased with an increase in reserved volume and that this increase came at a constantly decreasing rate. With the influence of reserved volume held constant, the increment decreased as the size of the average tree increased, and this decrease came at a constantly decreasing rate. This naturally follows from the fact that with a given reserved volume the amount of growing surface increases with a decrease in the average size of the trees left after cutting. Furthermore, as the size of the average tree decreases the average age also decreases, and from the nature of the curve of growth with time it follows that, in the average, more growth can be expected from a given volume of young trees than for the same volume of older trees.

The average mortality per acre for a period of 5 years was found to be 108.8 board feet with a standard error of 18.4 board feet. No significant relationship was found between mortality and years after cutting. There was, however, evidence of correlation between mortality and the two independent factors, reserved volume and volume of the average tree left after cutting, since an increase in either variable resulted in an increase in mortality. This relationship was not strong enough, however, to indicate definitely whether or not it was proportional.

U. S. GOVERNMENT PRINTING OFFICE: 1935